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ORIGINAL ARTICLES

STUDIES ON *BEMISIA GOSSYPIPERDA*, M. & L.

No. 3* SEASONAL ACTIVITIES OF *BEMISIA GOSSYPIPERDA*, M. & L. (THE WHITE-FLY OF COTTON) IN THE PUNJAB

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(With two text-figures)

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* No. 1. A Preliminary Note on the White-fly of Cotton in the Punjab. *Agric. J. Ind.*, Vol. XXV, Part VI, Nov. 1930.

No. 2. Observations on the Life-history, Bionomics and Control of the White-fly of Cotton (*Bemisia gossypiperda*, M. & L.). *Ind. J. Agric. Sci.* Vol. III, Part V, Oct. 1933.

I. Introductory

Since *B. gossypiperda* can obtain subsistance from a variety of plants, belonging to over thirteen different families [Husain and Trehan, 1933 ; Massop, 1932], it is obvious that any enquiry into the bionomics of this White-fly cannot be complete without an appreciation of its activities on its alternative hosts. Moreover, the intensity of infestation on cottons depends, besides other factors, on the survival of the pest during the season when the cotton crop is not growing, and also on its initial population at the beginning of the cotton season. Evidently, therefore, the possibility of an effective prevention by the ' safe disposal ' of the alternative host-plants cannot be ignored. Therefore, observations were made during 1932 and 1933 on the incidence of *B. gossypiperda* on its various host-plants, with a view to determine its preference for any of them and to discover the relative importance of the different hosts as sources of infection for the subsequent cotton crop.

The Indian Central Cotton Committee financed a scheme of research on the White-fly of cotton and this paper deals with a portion of the work done under this scheme. The generosity of the Indian Central Cotton Committee is gratefully acknowledged.

II. Technique

Entire plants, or where that was not practicable, entire leaves* of the various food-plants of *B. gossypiperda*, wild and cultivated, collected from within a radius of about two miles from the Agricultural Research Institute, Lyallpur, were examined and White-fly infestation worked out in relation to leaf area. During these investigations about 27,594 leaves of cottons with an area of 139,082 square inches, and 20,949 leaves of other host-plants, 69,339 square inches in area, were examined. For the study of host preference for oviposition, experiments were carried out in the laboratory in small muslin cages.

III. Migrations of the pest

Since the most important cultivated food-plants of *B. gossypiperda* are annuals, and the wild food-plants are mostly herbaceous perennials, the pest must of necessity live on its different hosts during different parts of the year. Irrespective of any preference for any of its food-plants, the density of White-fly population on a particular host is influenced to a large extent by its proximity to the cotton fields infested by the White-fly, protection from wind and adequate humidity. Another factor, perhaps of the greatest significance, is the stage of growth of the plant. Further, as stated by Nath and Suryanarayana [1929], Metcalf and Flint [1932], Delong [1934], Hargreaves [1934], Jackson [1934] and others, the radical changes in the composition of the cell sap in the leaves of the plants at different periods of growth, indicating the physiological state of the host, may be of the greatest importance. These changes may be responsible for bringing about seasonal migrations of *B. gossypiperda* from host to host.

* In case of such plants as cucurbits, a number of leaves could only be examined.

Whatever may be the cause of these fluctuations in population, this pest has been observed to undergo, in general, three phases of migration during the course of a year.

(*1*) *Migration from cotton to the winter alternative host-plants*

During November when the cotton crop is maturing and the leaves are drying up and shedding, the population of White-fly falls greatly on this plant, and its infestation starts on the alternative host-plants such as, rape (*Brassica napus*), cauliflowers (*Brassica oleracea*), turnips (*Brassica rapa*) and potatoes (*Solanum tuberosum*) among the cultivated plants, and *Sonchus* spp., *Euphorbia* spp., and *Convolvulus arvensis*, etc., among the commoner of the weeds.*

From December onward the number of adults falls considerably but the immature stages, *viz.*, eggs and nymphs, remain on these alternative hosts throughout the winter (Table 1).

It will not be out of place to mention that at times the intensity of infestation on certain of the host-plants increases sporadically. For instance, during November and December 1932 the intensity of attack on *Solanum tuberosum* and *Duranta plumeri* was very high and was much severer than on any of the other host-plants. Further, it has also been observed that the intensity of attack may vary, on the same host, growing in two different situations in the same locality, in which case, probably, the microclimatic factors play an important part.

The adults commence emerging from about the end of January and multiply once again on the winter host-plants such as *Brassica napus*, *Brassica oleracea*, *Brassica rapa*, *Raphanus sativus*, *Sonchus* and *Euphorbia* spp. and *Convolvulus arvensis*. The population thus increases for the future spread of the pest.

(*2*) *Migration from the winter alternative host-plants to the spring alternative host-plants*

By the end of March the various cultivated cucurbits, namely, *Cucumis melo* ; *Citrullus vulgaris* ; *Cucumis sativus*, *Lagenaria vulgaris* and *Luffa acutangula*, etc., come up and the White-fly migrates from the winter alternative food plants to these spring hosts, where rapid multiplication takes place. Besides these, the infestation at this stage is also observed on *Nicotiana tabacum*, *Hibiscus esculantus* and *Althea rosea*, and from April onward the ratoon cottons are also severely infested. By the time the infestation has increased on the above-named host-plants, in general, the infestation on weeds has fallen considerably.

It has been observed, that, during April and May the ratoon cottons and the cucurbits, in general, and melons in particular, form the most important breeding

* The pest has also been recorded on various other plants but infestation on these is very light.

centres. Hence, the ratooning of cotton and growing of melons mixed with cotton, or close to cotton fields, is likely to lead to a severe White-fly attack to the next cotton crop.

(3) Migration from the spring alternative host-plants to the cottons

The White-fly makes its appearance on the new cottons early in May, that is, soon after the crop has germinated, but the attack at that stage is extremely low as compared to the other host-plants. From June, however, partially because of the migration from the alternative host-plants, but mostly on account of the rapid multiplication of the pest, the intensity of attack increases enormously on the new cotton crop. During this period the attack on the cucurbits declines, but continues to be high on the ratoon cottons uptil the middle of July. In 1932, both the new and ratoon cottons were allowed to grow together in the same plot and relative incidence of the White-fly attack was worked out from June to the end of August. During June and upto the 5th of July the infestation was higher on the ratoon cotton and after that it was higher on the new crop (Table below).

Relative incidence of White-fly attack on the ratoon and fresh cotton crop in 1932

Host-plants	Average number of immature stages of White-flies per square inch of leaf area									
	18-VI	26-VI	5-VII	13-VII	20-VII	30-VII	6-VIII	12-VIII	19-VIII	26-VIII
Ratoon 4 F cotton	6·8	6·8	9·2	10·2	16·4	9·6	5·7	3·1	2·1	...
New 4 F cotton	2·7	5·4	7·1	10·2	19·1	16·9	9·7	4·8	2·4	1·6

These observations lead us to the conclusion that cucurbits and ratoon cottons act as the immediate and direct sources of White-fly infestation for the new cotton crop. The winter crops and particularly the weeds which harbour the immature stages of this pest during the winter are also of very great importance. Hence the value of clean cultivation and destruction of weeds is quite obvious.

The seasonal history of the pest in relation to some of the more important host-plants has been tabulated in Table II.

IV. Cross-inoculations

To verify the status of the various hosts as the true food-plants of *B. gossypiperda*, series of cross-inoculations were tried.* For each of these experiments, adults of White-fly were collected from definite host-plants and liberated on to

*A few of these crosses had been tried previously and the results confirmed. *Ind. J. Agric. Sci*, Vol. III, Part V, October 1933, p. 701-753.

the other host-plants, which had been grown under glass chimneys to keep them free of infestation. The following cross-inoculations have been tried successfully :—

Adults of *B. gossypiperda* collected from	Life-cycle completed on
Cotton	Cucurbits ; *Sonchus arvensis* ; *Achyranthus aspera*, *Brassica oleracea* ; *Digera arvensis* ; *Chaenopodium murrale* and *Raphanus sativus*.
Convolvulus arvensis . .	Cotton ; *Solanum nigrum* ; *Sonchus arvensis* ; *Brassica oleracea*.
Cucurbits	Cotton.
Althea rosea. . . .	Cotton.
Lycopersicum esculentum .	Cotton ; *Achyranthus aspera*.
Ipomea sp.	Cotton.
Brassica oleracea . .	Cotton ; *Withania somnifera* and *Brassica rapa*.
Solanum melongena . .	Cotton ; *Chaenopodium murrale*.
Solanum nigrum . . .	*Brassica oleracea*.
Sonchus arvensis . . .	*Brassica rapa*.
Achyranthus aspera . .	*Brassica rapa*.
Ratoon cotton . . .	*Raphanus sativus*.

These observations have shown that the plants cited above are the actual host-plants of the White-fly of cotton. Further, it has also been noticed that even *B. gossypiperda* is not so unorthodox as to feed on all and sundry plants. Repeated attempts were made to obtain eggs of this White-fly on leaves of *chari* (*Andropogon Sorghum*), but without success.

V. Host preference for oviposition

In order to study host preference, observations were made as to the relative oviposition on *Gossypium* spp., *Althea rosea*, *Lagenaria vulgaris*, *Cucumis melo*, *Luffa acutangula* and *Cucumis sativus* from May to July, and on *Gossypium* spp. *Brassica rapa*, *Raphanus sativus*, *Brassica oleracea*, *Solanum melongena*, *Brassica napus*, *Solanum tuberosum* and *Lycopersicum esculentum* from October to December, *i.e.*, corresponding to the periods of migration in nature from the spring alternative host-plants to cotton and from cotton to the winter host-plants respectively. The plants under observation were sown together in small beds and were enclosed, soon after germination, in small muslin cages, 2 ft. × 2 ft. × 1½ ft. For each observation about five hundred adults were collected in a glass chimney, which was then placed in the middle of the cage. The mouth of the chimney was covered over with a cardboard and the adults were allowed to escape through a small hole. After twenty-four to forty-eight hours' exposure, the plants were removed and all the leaves examined under a binocular and the eggs laid recorded. For comparison, the data were correlated with the leaf area examined. During May, relatively more eggs were laid on melons (*Cucumis melo*) than on cotton, but during July, oviposition increased considerably on cottons, var. Mollisoni

leading. From October onward, oviposition decreased on cotton and increased on *Brassica* spp., *Solanum tuberosum* and other alternative host-plants (Tables III and IV) and Fig. 1.

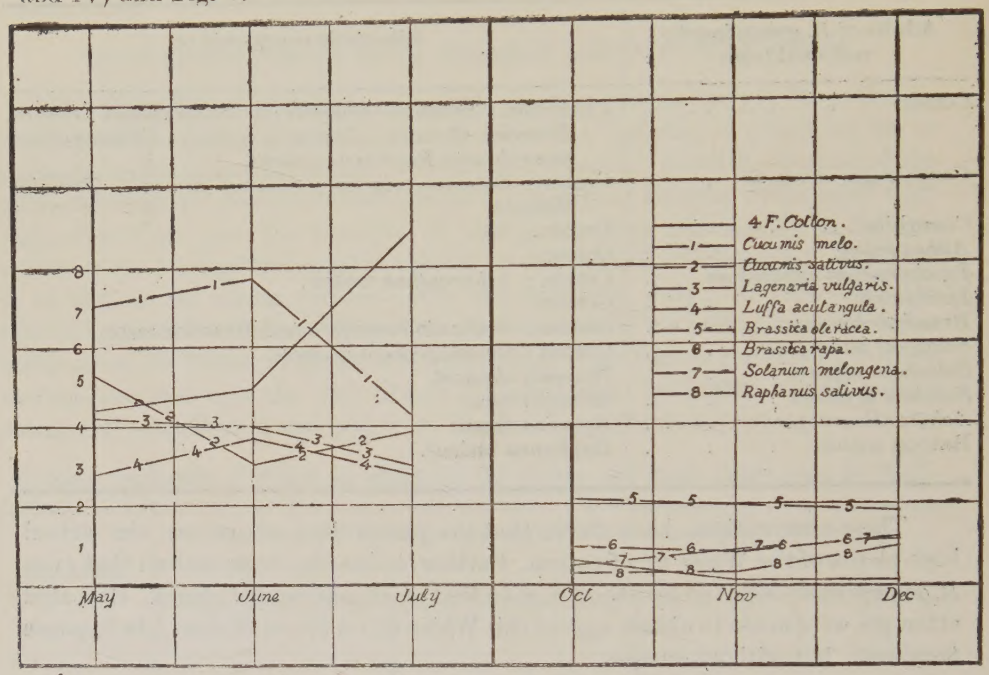

Fig. 1. Showing relative oviposition of *B. gossypiperda* on cotton and other alternative host plants.

VI. Period of severest infestation on cotton

A study of the life-history of *B. gossypiperda* has shown that the duration of its life-cycle from April to September is, on an average, about eighteen days. The shortest duration of fourteen days was noticed during August, while the longest duration of eighty-eight days in the laboratory, extended from November to February. The most favourable period of quick multiplication of the pest, therefore, coincides with the period of germination and growth of the cotton crop.

To determine the severity of White-fly attack and relative infestation on different varieties of cotton, observations were continued from 1931 to 1933 on six varieties, namely, Mollisoni, Sanguinum and Rosea representing *desi*, and 4 F, 285 F and 289 F, representing the American types. For each observation all the leaves on individual plants of each type were examined and the White-fly infestation was worked out in terms of leaf area. Some striking differences have been observed among these varieties regarding the initial attack. To start with, the intensity of the White-fly attack is comparatively severer on the *desi* (indigenous) varieties—Mollisoni leading, but from about the middle of August the infestation increases relatively on the American varieties. During 1933, however, the intensity of

attack, in general, was considerably low, hence the difference in attack on the various varieties was not so marked. The data collected with regard to the relative infestation on Mollisoni and 4 F varieties are presented in Table V and Fig. 2. It has been established that the period of the severest attack on cottons extends from June to August, after which the infestation, as a rule, declines abruptly.

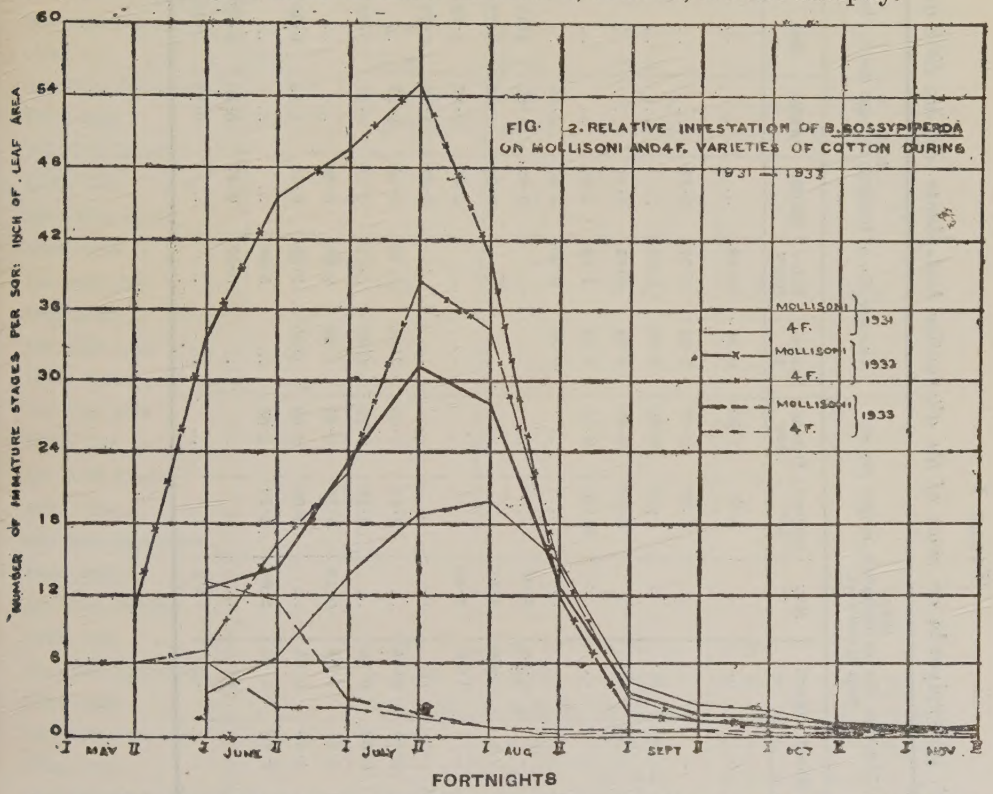

Fig. 2. Relative infestation of *B. gossypiperda* on Mollisoni and 4 F varieties of cotton during 1931-1933.

VII. Summary

A study of the seasonal activities of *B. gossypiperda* shows that there are three phases of migration of the pest in the course of a year; (1) from cotton to the winter alternative hosts, (2) from there to the spring alternative hosts and (3) from the latter back again to cotton. Cucurbits and ratoon cotton have been regarded as dangerous sources of infection for the new cotton. Period of quick multiplication of the pest coincides with that of germination and growth of the cotton crop. Severity of attack on cotton extends from June to August. The attack on ratoon cotton continues to be the severest till about the middle of July. Preference for oviposition has been observed for certain host-plants during different periods. Infestation on weeds during the off-season of cotton has indicated that the pest might be checked by practising clean cultivation and removal of weeds.

TABLE I

Comparative infestation of B. gossypiperda *on some of the alternative host-plants during 1932 and 1933*

Host-plant	Common name	1932 Average No. of immature stages per sq. inch of leaf area					1933 Average No. of immature stages per sq. inch of leaf area						
		March	April	May	November	December	January	February	March	April	May	November	December
Brassica napus	Indian rape (*Toria*).	...	...	...	3·60	2·50	0·38	0·59	...	...	...	0·67	0·57
Brassica oleracea	Cauliflower	...	...	...	2·28	0·76	0·24	0·77	0·17	...	...	0·45	0·55
Brassica rapa	Turnips	...	...	...	0·32	0·62	0·35	0·37	...	...	...	0·39	0·16
Raphanus sativus	Radish	...	...	...	0·59	0·46	0·34	0·25	0·11	...	...	0·18	0·16
Solanum tuberosum	Potato	...	...	...	37·10	6·10	1·14	1·60	0·43	...	...	0·84	0·66
Lycopersicum esculentum	Tomato	...	...	...	...	...	0·33	0·06	0·46	0·45	...	0·41	0·40
Cucumis melo	Melon	...	2·10	6·60	...	...	...	...	0·62	1·61	2·15	...	...
Cucumis sativus	Cucumber	...	1·04	4·51	...	...	...	...	0·26	1·43	2·72	...	...
Lagenaria vulgaris	Gourd	...	1·11	2·40	...	...	...	...	0·29	0·75	1·32	...	...
Hibiscus esculentus	Lady's finger	...	...	...	...	...	...	...	0·33	1·26	0·78	...	...
Convolvulus arvensis	Bind Weed (*Lehli*)	0·48	0·44	...	2·95	1·05	1·41	1·72	0·10	0·27	...	0·56	1·30
Withania somnifera	*Asgandh* (*Aksin*)	0·52	0·16	...	0·20	0·32	0·40	0·41	0·41	0·21	0·28	0·25	0·16
Achyranthus aspera	*Puthkanda*	0·91	0·78	0·44	0·19	1·31	1·22	0·95	0·39	...	...	0·51	0·36
Sonchus arvensis	Sow thistle	1·50	0·54	...	2·68	2·66	1·50	3·55	6·53	1·15	0·25	1·90	2·60
Euphorbia hypercifolia	*Hazar Dana* (*Dodhak*).	2·70	1·40	2·84	2·70	1·63	...	5·96	...	...	...	0·41	0·87
Solanum nigrum	Black night shade (*Makoh*).	...	...	...	...	...	...	0·57	0·47	0·36	0·23	...	0·42
Ratoon cotton	...	...	...	4·2	...	...	...	...	...	0·38	2·44	...	...

TABLE II

Annual cycle of the distribution of various stages of the White-fly on different host

Duration	*Gossypium* spp. all varieties (New cotton)	*Gossypium* spp. (Ratoon cotton)	*Cucurbit* spp.	*Brassica* spp.	*Nicotiana tabacum*	*Solanum tuberosum*	*Solanum melongena*	*Cyamopsis psoral-oides*	*Solanum nigrum*	*Hibiscus esculentus*
1st—15th January . .	E, N.	...	...	E, N.	...	E, N.	...	...	E, N.	...
16th—31st ,, . .	N, P.	...	...	N, P.	...	N, P.	...	...	N, P.	...
1st—15th February . .	...	...	...	A, N, P.	...	A, N, P.	...	...	A, N, P.	...
16th—28th ,, . .	...	...	...	A, E, N, P.	...	A, E, N, P.	...	...	A, E, N, P.	...
1st—15th March . .	...	...	A	A, E, N, P.	A, E, N.	...	A	...	A, E, N. P.	A.
16th—31st ,, . .	...	...	A, E, N.	,,	A, E, N, P.	...	A, E, N.	...	...	A, E, N.
1st —15th April. . .	...	A, E.	A, E, N, P.	...	,, .	...	A, E, N, P.	...	...	A, E, N, P.
16th—30th ,, . . .	A	A, E, N, P.	,,	...	,, .	...	,,	A, E.	...	,,
1st—15th May . . .	A, E, N, P.	,,	,,	...	,, .	...	,,	A, E, N, P.	...	,,
16th—31st ,, . .	,,	,,	,,	...	...	...	,,	,,	...	,,
1st—15th June . . .	,,	,,	,,	...	... .	...	,,	,,	...	,,
16th—30th ,, . . .	,,	,,	,,	...	...	...	,,	,,	...	,,
1st—15th July . . .	,,	...	...	...	...	...	...	,,	...	...
16th—31st ,, . . .	,,	...	...	...	...	...	...	,,	...	...
1st—15th August . .	,,	...	...	...	...	...	...	...	...	...
16th—31st ,, . .	,,	...	...	...	...	...	...	...	...	...
1st—15th September . .	,,	...	...	...	...	...	...	...	...	...
16th—30th ,, . .	,,	...	...	...	...	...	...	...	...	...
1st—15th October . .	,,	...	...	A, E.	...	A, E.	...	...	...	...
16th—31st ,, . .	,,	...	...	A, E, N.	...	A, E, N.	...	...	A. E.	...
1st—15th November . .	,,	...	...	A, E, N, P.	...	A, E, N, P.	...	...	A, E, N.	...
16th—30th ,, . .	,,	...	...	A, E, N, P.	...	A, E, N, P.	...	...	A, E, N, P.	...
1st—15th December . .	A, E, N.	...	...	A, E, N.	...	A, E, N.	...	...	A, E, N.	...
16th—31st ,, . .	A, E, N	...	...	A, E, N.	...	A, E, N.	...	...	A, E, N.	...

N. B.—1. A—Adult. E.—Egg. N.—Nymph. P.—Pupa.
2. *Gossypium* spp. include both *desi* and American cottons.
3. *Cucurbit* spp. include *Cucumis melo ; Cucumis sativus ; Lagenaria vulgaris ; Cucumis pubescens.*
4. *Euphorbia* spp. include *Euphorbia prostata ; Euphorbia pilulifera ; Euphorbia hypercifolia.*
5. *Brassica* spp. include *Brassica napus ; Brassica oleracea ; Brassica rapa ; Brassica campestris.*
6. *Sonchus* spp. include *Sonchus arvensis* and *Sonchus oleracea.*

-plants

Lycopersicum esculentum	*Sonchus* spp.	*Withania somnifera*	*Convolvulus arvensis*	*Euphorbia* spp.	*Achyranthus aspera*
E, N.	E, N.	E, N.	E, N.	E, N.	E, N.
N, P.	N, P.	N, P.	N, P.	N, P.	N, P.
A, N, P.	A, N, P.	A, N, P	A, N, P.	A, N, P.	A, N, P.
A, E, N, P.	A, E, N, P.	A, E, N, P.	A, E, N, P.	A, E, N, P.	A, E, N, P.
,,	,,	,,	,,	,,	,,
,,	,,	,,	,,	,,	,,
,,	,,	,,	,,	,,	,,
,,	,,	,,	,,	,,	,,
...	N, P.	N, P.	N, P.	N, P.	N, P.
...	...	...	...	...	...
...	...	...	...	...	...
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...	...	...	...	...	...
...	A, E.	A. E.	A, E.	A, E.	A, E.
...	A, E. N.	A, E. N.	A, E,N.	A, E, N.	A, E, N.
A, E.	A, E, N, P.	A, E, N, P,	A, E, N, P.	A, E, N, P.	A, E, N, P.
A, E, N.	A, E, N.	A, E, N.	A, E, N,	A, E, N.	A, E, N.
A, E, N.	A, E, N.	A, E. N.	A, E, N.	A, E, N.	A, E, N.

TABLE III

Comparative oviposition of B. gossypiperda, *on cotton and al*

Host-plants	May			June			July			
	Leaf area sq. in.	No. of eggs laid	Eggs per sq. in.	Leaf area sq. in.	No. of eggs laid	Eggs laid per sq. in.	Leaf area sq. in.	No. of eggs laid	Eggs laid per sq. in.	
Gossypium sp. 4 F.	87·64	379	4·4	149·63	757	5·0	120·34	974	8·9	*Go*
Gossypium sp. Mollisoni	76·40	446	5·8	103·02	725	7·3	76·71	847	11·4	*Br*
Althea rosea	57·04	84	1·4	26·12	72	2·7	7·51	16	2·1	*Ra*
Lagenaria vulgaris	153·37	645	4·2	166·49	669	4·0	114·13	347	3·0	*Br*
Luffa acutangula	165·43	468	2·8	133·69	498	3·7	73·04	208	2·8	*So*
Cucumis sativus	101·93	550	5·3	157·55	498	3·1	123·64	487	3·9	*Br*
Cucumis melo.	141·72	1,017	7·1	101·79	797	7·8	107·59	473	4·3	*So*
										Ly

TABLE IV

Showing the above figures arranged in order of preference regardi

Host-plants	May		June		July		
	In order of leaf area	In order of No. of eggs	In order of leaf area	In order of No. of eggs	In order of leaf area	In order of No. of eggs	
Gossypium sp. 4 F.	V	(6)	III	(2)	II	(1)	*Go*
Gossypium sp. Mollisoni	VI	(5)	V	(3)	V	(2)	*Br*
Althea rosea	VII	(7)	VII	(6)	VII	(7)	*Ra*
Lagenaria vulgaris	II	(2)	I	(4)	III	(5)	*Br*
Luffa acutangula	I	(4)	IV	(5)	VI	(6)	*Sol*
Cucumis sativus	IV	(3)	II	(5)	I	(3)	*Br*
Cucumis melo.	III	(1)	VI	(1)	IV	(4)	*So*
							Ly

ternative host-plants growing under the same cage

Host-plants	October			November			December		
	Leaf area sq. in.	No. of eggs laid	Eggs laid per sq. in.	Leaf area sq. in.	No. of eggs laid	Eggs laid per sq. in.	Leaf area sq. in.	No. of eggs laid	Eggs laid per sq. in.
ssypium sp. 4 F	67·44	39	0·57	60·70	7	0·11	81·62	9	0·11
rassica rapa	44·00	42	0·95	71·83	56	0·77	275·06	332	1·20
yphanus sativus	149·65	59	0·32	235·18	73	0·31	657·59	655	0·99
rassica oleracea	52·31	113	2·16	142·15	283	1·99	369·48	663	1·79
lanum melangena	35·33	16	0·45	23·99	19	0·8	14·62	17	1·16
rassica napus	...	...	...	...	...	...	54·24	178	3·28
lanum tuberosum	...	...	...	...	...	...	41·85	106	2·53
icopersicum esculentum	...	...	...	...	...	...	62·22	217	3·48

ng the available leaf area and the density of population

Host-plants	October		November		December	
	In order of leaf area	In order of No. of eggs	In order of leaf area	In order of No. of eggs	In order of leaf area	In order of No. of eggs
ypium sp. 4 F	II	(4)	IV	(5)	IV	(8)
sica rapa	IV	(3)	III	(3)	III	(3)
hanus sativus	I	(2)	I	(2)	I	(2)
sica oleracea	III	(1)	II	(1)	II	(1)
num melongena	V	(5)	V	(4)	VIII	(7)
sica napus	...	...	...	...	VI	(5)
num tuberosum	...	...	...	...	VII	(6)
opersicum esculentum	...	...	...	...	V	(4)

TABLE V

Relative infestation of B. gossypiperda, *on Mollisoni and 4-F varieties of cotton*

Variety	Year	Average number of immature stages per square inch of leaf area													
		May Fortnight		June Fortnight		July Fortnight		August Fortnight		September Fortnight		October Fortnight		November Fortnight	
		I	II	I	II	I	II	I	II	I	II	I	II	I	II
Mollisoni	1931	...	...	12·3	14·1	23·0	30·9	27·8	12·4	3·2	1·5	1·3	0·6	0·4	0·3
4-F.		...	...	3·3	6·5	13·6	18·7	19·7	14·5	4·3	2·4	2·0	0·9	0·7	0·2
Mollisoni	1932	...	10·8	33·5	45·5	49·3	55·1	40·4	11·7	1·6	1·1	0·7	0·3	0·5	0·5
4-F.		6·1	6·1	7·1	16·1	22·2	38·4	34·3	14·0	3·0	1·0	0·6	0·3	0·2	0·3
Mollisoni	1933	...	...	12·8	11·4	2·9	1·7	0·5	0·4	0·3	0·4	0·1	0·1	0·3	0·8
4-F.		...	...	6·1	2·2	2·2	1·3	0·5	0·1	0·2	0·2	0·1	0·2	0·1	0·1

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STUDIES ON THE ROOT-ROT DISEASE OF COTTON IN THE PUNJAB

II. SOME STUDIES IN THE PHYSIOLOGY OF THE CAUSAL FUNGI

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(With two text-figures)

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PREFACE

An investigation of the root-rot disease of cotton in the Punjab was taken in hand by Dr. R. S. Vasudeva, Assistant Cotton Mycologist, appointed for the purpose in the Scheme financed by the Indian Central Cotton Committee since 1932.

During the first three years of the Scheme, most of the time has been devoted to the discovery of causal organisms of the disease. The results definitely establish that the disease is caused mainly by two fungal parasites, namely *Rhizoctonia bataticola* and *Rhizoctonia solani*. A paper giving an account of the experimental work carried out in this connection leading to definite conclusions with regard to the pathogenic nature of the disease was published in this *Journal* (1935). In the present paper, which is in continuation of the previous one, is described how the various fungi react with regard to parasitism and growth on different media alone and in combination with some non-parasitic fungi that were isolated from the roots of cotton plants affected by the disease. The know-

ledge gained on the pathology of the causal organisms of the disease is likely to indicate the way in which the parasites bring about injury to the roots of the host-plants.

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I. FUNGI STUDIED

The investigation reported in the paper was carried out with the following fungi isolated from the roots of cotton plants affected by the root-rot disease :—

1. A fungus provisionally *called *Rhizoctonia bataticola.*
2. *Rhizoctonia solani.*
3. Strain A of *Fusarium solani.*
4. Strain B of *Fusarium solani.*
5. A species of *Alternaria.*
6. A species of *Helminthosporium.*
7. *Fusarium species* (15).
8. *Fusarium species* (17).

Before commencing the work, the purity of the fungi was ensured by taking a single hyphal tip in each case, after the method described by Brown [1924] and stock cultures were maintained in tubes of potato extract agar.

II. MEDIA EMPLOYED

The following media with or without agar were used :—

1. Glucose peptone—

Glucose	10 grms.
Peptone	2 „
Potassium dihydrogen-phosphate	1 grm.
Magnesium sulphate	0·5 „
Distilled water	1,000 c. c

2. Brown's synthetic solution.
3. Potato extract—

Peeled potatoes	20 grms.
Distilled water to make up	1,000 c. c.

4. †Cotton root extract—

Cotton roots	200 grms.
Distilled water to make up	1,000 c. c.

*The systematic position of this fungus has not yet been exactly determined. It will be given in a later publication.

† Cotton roots or leaves were cut into small pieces, covered with distilled water and autoclaved at 15 lbs. pressure for one hour and passed through muslin. The extract thus obtained was made up to 1,000 c.c. by adding distilled water.

5 Cotton leaf extract—

Cotton leaves	200 grms.
Distilled water to make up	1,000 c. c.

6. Richards' agar.

7. Cotton root synthetic.

As the cotton root extract, though quite suitable for culture of the two *Rhizoctoniaspecies*, is liable to vary in composition ; it was thought that if a synthetic substitute could be found it would prove of great advantage and the results would be strictly comparable. In proceeding to evolve such a medium, a start was made by analysing chemically, roots of *desi* cotton (Mollisoni 15). The roots belonged to the plants which were about five months old. The average composition of the roots thus analysed is given below :—

Moisture, 65·00 per cent.	Fat	0·37 per cent.
	Protein	1·45 per cent.
Dry matter, 35·00 per cent.	N-free extract	10·49 per cent.
	Fibre	21·05 per cent.
	Ash	1·64 per cent.

As regards the constituent of ash, 1·64 grms. of it contain :—

Fe_2O_3	0·02 grm.
AI_2O_3	0·02 ,,
Ca O	0·25 ,,
Mg O	0·06 ,,
Mn_3O_4	0·01 ,,
Na_2O	0·02 ,,
K_2O	0·63 ,,
P_2O_5	0·08 ,,
S O_4	0·16 ,,
Insoluble residue	0·22 ,,

A decoction of 200 grms. of cotton roots in the litre of water contains the following :—

2·90 grms. nitrogenous substance.
0·74 grm. fat.
20·98 grms. nitrogen-free extract.
42·10 grms. fibre.
3·28 grms. ash.

In preparing the solution, the fat and fibre constituents of the organic part were ignored, as were also the sodium and aluminium constituents of the ash. The latter two were omitted as there is a strong evidence that these elements in general are not essential for fungal growth.

The remaining constituents of the cotton root extract were then added in the following form and proportion. The proportion was calculated to give as closely

as possible the constituents and quantities required according to the analytical data given above.

Glucose	20 grms.
Peptone	2·9 „
K_3PO_4	1·9 „
*$Ca_3(PO_4)_2$	0·9 „
$MgSO_4$	0·4 „
NaCl	0·6 „
$FeCl_3$	A trace.
Distilled water to make up	1,000 c.c.

This medium has proved more suitable for the growth of the two *Rhizoctonia* spp. than the other artificial media employed, as the growth on this synthetic medium is comparable and almost identical with that obtained on the decoction of the natural host.

III. Cultural characters of the two *Rhizoctonia* spp.

The fungi were grown on Brown's agar, Richards' agar, cotton leaf and root extracts, and cotton root synthetic at a temperature of 20°C., 25°C., 30°C., 35°C., 40°C. and 45°C., respectively. The plates used were of uniform diameter and into each was poured 50 c.c. of the medium in order to maintain uniformity in depth. These experiments were run in quadruplicate.

* $Ca_3(Po_4)_2$ is very slightly soluble in water and may be omitted without any appreciable difference in growth.

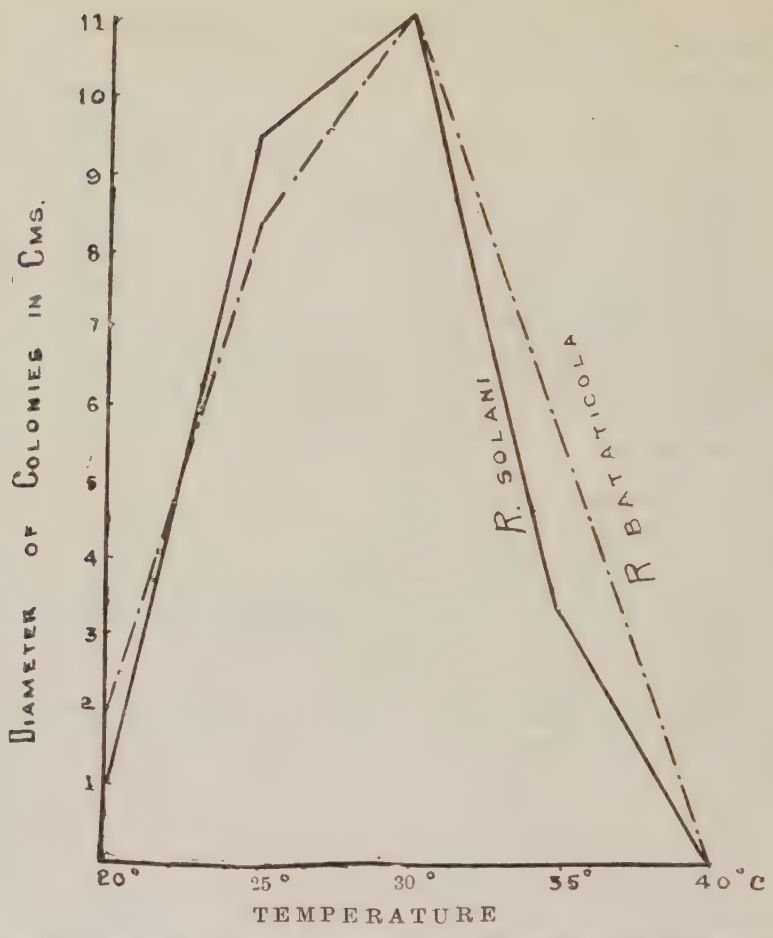

Fig. 1. Showing effect of temperature on rate of growth of *R. bataticola* and *R. solani* on cotton root synthetic agar (3 days' growth).

The relation between temperature and linear growth three days after inoculation is shown in Fig. 1 which gives the rate of spread of *Rhizoctonia bataticola* and *Rhizoctonia solani* on cotton root synthetic over a series of temperatures.

Both are fairly fast growing fungi with optimum growth at 30°C. Above 30°C. there is a fall in the rate of growth and a cessation occurs at 40°C. on all the media with the exception of Brown's agar on which *Rhizoctonia bataticola* exhibited slight growth even at 40°C. But no growth occurred at 45°C. even on this medium.

Rhizocotonia solani produces plenty of profuse white aerial mycelium which turns light brown with age. There are plenty of white aggregations of hyphae which later form dark brown hard sclerotia.

Rhizoctonia bataticola produces a white colony with plenty of aerial mycelium which turns dark grey with age and has numerous tiny dark sclerotial bodies. It does not produce pycnidia or spores of any kind.

IV. Growth behaviour of the pathogene under different conditions of nutrition

The growth behaviour of the two *Rhizoctonia* spp. was studied on cotton root synthetic solutions. The importance of each constituent of the synthetic solution was determined by preparing solutions lacking in that constituent. The two fungi were grown in 100 c.c. Erlenmeyer flasks containing 30 c.c. of the medium. The experiment was carried out in triplicate.

The flasks were inoculated after sterilization in the usual way with cultures of the fungi growing on potato-extract agar. Great care was taken and an inoculum of almost uniform size was introduced into each flask. The hydrogen-ion concentrations of uninoculated media were determined by Clark's method soon after sterilization. The dry weight of the fungal mats and the pH values of the stales were determined after the fungi had grown in cultures for twenty-four days. The fungal material was collected on filter papers by means of a Buchner filter, dried in an oven, cooled in a calcium chloride desiccator, and weighed.

The results of the experiment are given in Table I.

Table I

Weights of the mycelium of Rhizoctonia bataticola *and* Rhizoctonia solani *and pH of filtrates after twenty-four days' growth in various modifications of cotton root synthetic*

Medium	*Rhizoctonia bataticola*			*Rhizoctonia solani*		
	Average dry weight of mycelium in mg.	pH of un-inoculated control solution	pH of filtrates	Average dry weight of mycelium in mg.	pH of un-inoculated control solution	pH of filtrates
Complete cotton root synthetic	109	5·6	6·4	133	6·2	7·2
Complete medium—glucose .	20	8·0	8·0	11	7·8	8·2
Complete medium—peptone .	7	5·4	5·8	9	6·0	6·4
Complete medium—K_2PO_4 .	80	5·2	7·4	82	6·0	8·0
Complete medium—$Ca_3(PO_4)_2$	107	5·4	7·0	151	6·0	6·8
Complete medium—$MgSO_4$.	130	5·4	6·8	93	5·8	7·4
Complete medium—NaCl .	173	5·4	6·6	133	6·0	6·8
Complete medium—$FeCl_3$.	200	5·4	6·8	173	6·2	6·8
Complete medium—both K_2PO_4 and $Ca_3(PO_4)_2$.	63	4·8	5·0	83	5·4	6·0

(— lacking in).

As is clear from the above table, the omission of magnesium and sodium salts has little effect on the amount or kind of growth, whereas in the absence of iron salts the growth is comparatively better and more luxuriant. When sugar is omitted, the vigour of growth is considerably reduced. In the absence of peptone, the growth is reduced to a minimum and is in the form of a thin scanty film. Next in importance is the phosphate constituent in the absence of which the vigour of growth is also reduced.

Both the *Rhizoctonia* spp. tend to change the reaction of the medium and in all cases reduce the acidity of the solutions.

V. Effect of different pH concentrations on growth

For the investigation of influence on growth of media varying in pH value from 2·4 to 9·2, the two *Rhizoctonia* spp. were grown on glucose-peptone agar prepared with different degrees of acidity and alkalinity by addition of malic acid and sodium bicarbonate respectively. This medium is normally acid, 100 c.c. requiring approximately 0·07 grm. of sodium bicarbonate for neutralisation. Fifteen modifications of the medium were made up showing a range of acidity and alkalinity. The Petri dishes were inoculated in triplicates and the linear growth rates measured from day to day.

Fig. 2 shows diameters of colonies of *Rhizoctonia bataticola* and *Rhizoctonia solani* on media of different pH values.

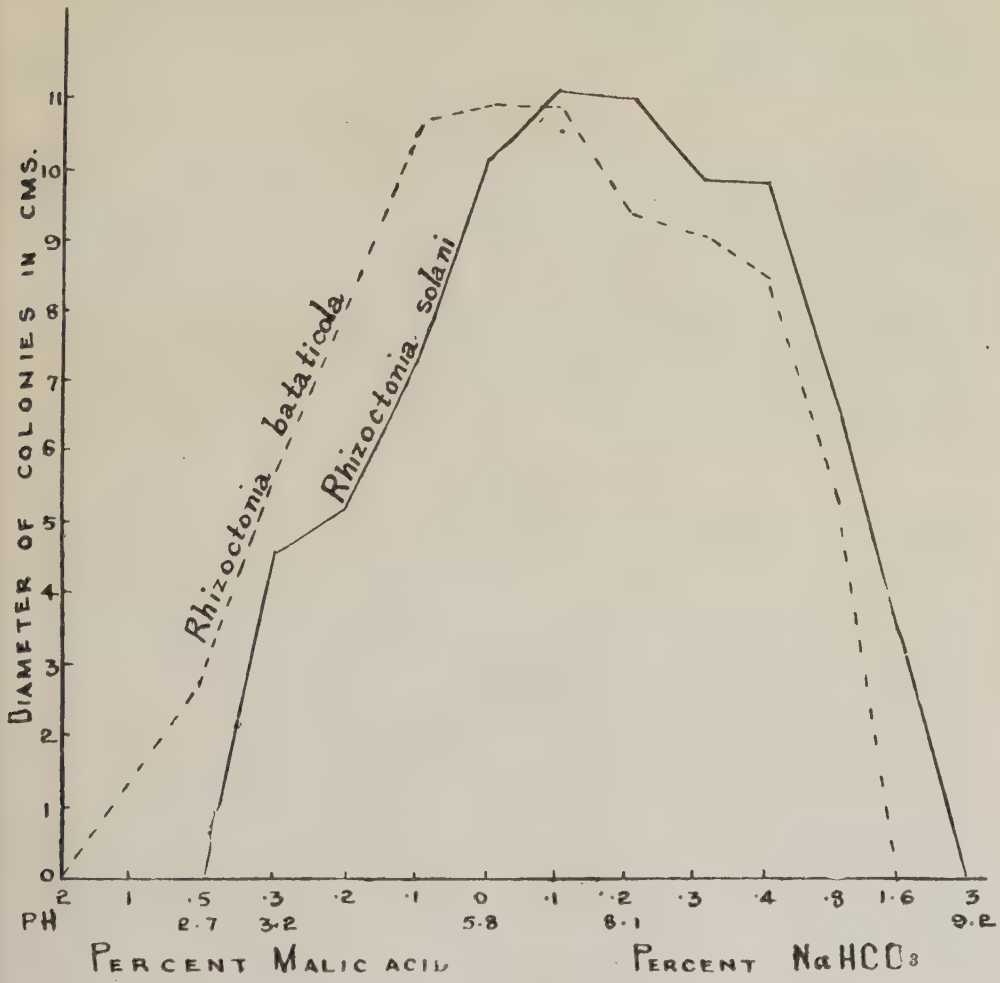

Fig. 2. Showing diameters of colonies (three days' growth) of *Rhizoctonia bataticola* and *Rhizoctonia solani* on media of different pH (Medium: Glucose-peptone).

It is evident from the data obtained that both the fungi have a very wide range of toleration to acidity and alkalinity, that is between pH 2·4 and 9·2 with fairly good growth between pH 3·2 and 8·5 and optimum near the neutral point. This experiment was repeated with cotton root synthetic as basal medium and similar results were obtained.

VI. Effect of carbon dioxide

The two *Rhizoctonia* spp. were grown in different concentrations of carbon dioxide, *e.g.*, twenty-five, fifty, seventy-five and hundred per cent on Richards' agar in quadruplicates. Controls were kept under ordinary atmospheric conditions.

The inoculated plates were kept in desiccators which were then sealed.

The carbon dioxide was let into the sealed chambers by creating the required amount of vacuum by adjustment of pressure by a manometer.

The results obtained by growing the fungi in normal atmosphere and in varying concentrations of carbon dioxide are given in Table II.

Table II

Effect of carbon dioxide on the growth of Rhizoctonia bataticola *and* Rhizoctonia solani

Fungus	Concentration of CO_2 per cent	Diameter of the colonies in centimeters (after 5 days' growth) Plate numbers				
		1	2	3	4	Average
Rhizoctonia bataticola	25	4·8	5·2	5·5	5·3	5·20
Rhizoctonia solani		1·5	1·2	1·6	1·3	1·86
Rhizoctonia bataticola	50	2·8	2·7	2·1	2·4	2·50
Rhizoctonia solani		0·0	0·0	0·0	0·0	0·00
Rhizoctonia bataticola	75	0·6	0·5	0·6	0·5	0·55
Rhizoctonia solani		0·0	0·0	0·0	0·0	0·00
Rhizoctonia bataticola	100	0·3	0·2	0·2	0·2	0·22
Rhizoctonia solani		0·0	0·0	0·0	0·0	0·00
Rhizoctonia bataticola	Control	10·4	10·2	10·4	10·6	10·40
Rhizoctonia solani		7·5	7·1	7·4	7·2	7·27

At fifty per cent concentration of carbon dioxide the growth of *Rhizoctonia solani* altogether ceased whereas a slight growth took place in the case of *Rhizoctonia bataticola* even at 100 per cent carbon dioxide concentration. At any rate marked reduction in growth was noticed in both the fungi even at twenty-five per cent carbon dioxide concentration. Though the growth is arrested at higher concentrations of carbon dioxide, yet the fungi are found to be viable even after forty-four days' exposure to pure carbon dioxide when normal atmospheric conditions are restored.

In another similar experiment twenty-five per cent and fifty per cent vacuum were created in sealed chambers but no carbon dioxide was introduced.

Here even at fifty per cent vacuum the difference in the amount of growth when compared with the controls was slight. This clearly shows that the depressing effect shown above, is only due to the introduction of carbon dioxide and not due to deficiency of ordinary atmosphere (oxygen).

VII. Effect of nitrogen

The effect of the atmosphere of nitrogen on the growth of *Rhizoctonia bataticola* and *Rhizoctonia solani* was studied by placing the inoculated Petri dishes in desiccators in which normal atmosphere had been replaced by nitrogen by the pyrogallic acid method. The difference between the rates of growth of the control and of those exposed to the atmosphere of nitrogen was very slight. But there was a marked difference in the amount of aerial mycelium. In the plates exposed to the atmosphere of nitrogen, the growth was thin, scanty and spreading with little aerial mycelium.

VIII. Thermal death point

In order to determine the thermal death point of the two *Rhizoctonia* spp. a suspension of the fungal mycelium was prepared in sterile water. Some narrow glass tubing which had been drawn into capillary form was cut into pieces two inches in length. Then each of these tubes was fused in the middle, thus making one inch capillary tubes sealed at one end. These tubes were then dropped into the water suspension of the fungal mycelium and exhausted under air pump. By this means, the capillary tubes got filled with the suspension. They were then placed in beakers of water at various temperatures ranging from 45 to 70° C. for five seconds ; capillary tubes so treated were then broken off at the ends and the contents collected on Richards' agar in Petri dishes. It was observed that *Rhizoctonia solani* failed to grow when exposed at 60° C. for five seconds but in the case of *Rhizoctonia bataticola* an exposure of five seconds at 68° C. was required to kill the fungus. A similar experiment to determine the thermal death point of the sclerotia of the two *Rhizoctonia* spp. was carried out but this revealed no difference between the thermal death point of the mycelium and the resting bodies.

Capillary tubes filled with a suspension of fungal mycelium of *Rhizoctonia bataticola* and *Rhizoctonia solani* were exposed to 0°C. The viability tests were carried out after exposure from 1 to 120 hours but the fungi were found to be viable.

IX. Effect of mixture of organisms on the activity of the two *Rhizoctonia* spp.

In a preceding paper of this series [Vasudeva ; 1935, 1] it was shown that the two *Rhizoctonia* spp. almost always appeared together when isolations were made

from the diseased roots of cotton and were the cause of root-rot disease of cotton in the Punjab. In addition several strains of *Fusarium*, a species of *Alternaria* and *Helminthosporium* were isolated.

The effect of the parasitic activity of the two *Rhizoctonia* spp. when occurring singly or in mixtures either with each other or with one of the other organisms mentioned was a matter which attracted considerable attention. The two strains of *Fusarium* which were later identified as those of *Fusarium solani*, a species of *Alternaria* and *Helminthosporium* and in addition two more strains of *Fusarium* did not produce the symptoms of root-rot disease.

Experiments were carried out with batches of cotton plants of 15 Mollisoni and 4 F varieties respectively.

The general plan of the experiment was to inoculate batches of cotton plants as follows :—

(*i*) A batch of cotton plants with parasitic fungus alone.

(*ii*) A batch of cotton plants with non-parasitic fungus.

(*iii*) A batch of cotton plants with active+inactive fungus.

(*iv*) A batch of cotton plants as controls.

The inoculations were made in a standardized way by exposing the roots and adding a uniform amount of inoculum by weight and covering up by means of the sterilized soil. In the case of the controls the fungal inoculum was not introduced.

In all the four batches uniform conditions were maintained throughout the experiment whether carried out in laboratory or in field.

In some experiments both the active fungi, *i.e.*, the two *Rhizoctonia* spp. were introduced together and in others either of them singly was used.

The results of a typical experiment in which the active organism used was *Rhizoctonia solani* are set out in Table III.

TABLE III

Variation in the parasitic activity of Rhizoctonia solani *in the presence of other organisms*

Inoculum	No. inoculated	No. wilted	Percentage of mortality
Rhizoctonia solani	10	2	20·00
Rhizoctonia solani+*Fusarium solani* (*A*) . .	6	2	33·33
Rhizoctonia solani+*Fusarium* sp. (15) . . .	8	8	100·00
Rhizoctonia solani+*Fusarium* sp. (17) . . .	4	4	100·00
Rhizoctonia solani+*Fusarium sol.* (*B*) . . .	6	2	33·33
Rhizoctonia solani+*Helminthosporium* sp. . .	6	3	50·00

Duration of the experiment was one week.

These are the results of an experiment carried out under controlled laboratory conditions. Controls in all cases remained unaffected. All the comparisons shown above indicate that the parasitic activity of *Rhizoctonia solani* is enhanced when it acts in combination with other fungi.

On repeating the experiment similar results were obtained. These results are in contrast with those obtained by Vasudeva [1930] for *Monilia fructigena* and *Botrytis cinerea* on apple, where the activity of the organisms was considerably reduced in mixed inoculations. But these are in accordance with Fawcett [1923] who obtained increased parasitic activity of *Pythiacystis citrophthora* in presence of a species of a *Fusarium* in the inoculum and with Vasudeva [1935, 2] who observed increased parasitic activity of *Rhizoctonia solani* in the presence of species *Alternaria* and *Helminthosporium* on turnips.

When *Rhizoctonia bataticola* is introduced into the inoculum in combination with the other fungi, *i.e.*, various strains of *Fusarium* species of *Helminthosporium* and *Alternaria*, there is no significant difference in the parasitic activity.

The same type of experiment was repeated using either of the *Rhizoctonia* spp. singly against both of them acting together. The results from six such experiments carried out in the laboratory are given in Table IV.

TABLE IV

The parasitic activity of the two Rhizoctonia *spp. when parasitising singly and jointly under controlled laboratory conditions*

Experiment	Inoculum	No. inoculated	No. wilted	Percentage mortality	Controls
I	*Rhizoctonia solani* . . .	15	3	20·00	13, all sound
	Rhizoctonia bataticola . .	15	5	33·33	
	R. solani+*R. bataticola* . .	12	12	100·00	
II	*Rhizoctonia solani* . . .	11	1	9·09	9, all sound
	Rhizoctonia bataticola . .	12	11	91·67	
	R. solani+*R. bataticola* . .	9	9	100·00	
III	*Rhizoctonia solani* . . .	13	3	23·08	11, all sound
	Rhizoctonia bataticola . .	11	9	81·82	
	R. solani+*R. bataticola* . .	11	9	81·82	
IV	*Rhizoctonia solani* . . .	11	2	18·18	11, all sound
	Rhizoctonia bataticola . .	10	1	10·00	
	R. solani+*R. bataticola* . .	12	8	66·67	
V	*Rhizoctonia solani* . . .	12	1	8·33	12, all sound
	Rhizoctonia bataticola . .	13	12	92·31	
	R. solani+*R. bataticola* . .	12	12	100·00	
VI	*Rhizoctonia solani* . . .	5	2	40·00	5 all sound
	Rhizoctonia bataticola . .	4	..	..	
	R. solani+*R. bataticola* . .	4	4	100·00	

The results shown above indicate that the parasitic activity of *Rhizoctonia solani* tends to increase when acting in combination with *Rhizoctonia bataticola*.

Increased parasitic activity is also shown by *Rhizoctonia bataticola* when parasitising in combination with *Rhizoctonia solani*. In the 3rd experiment however, its parasitic activity remains unchanged.

In conclusion I wish to place on record my thanks to Professor Rai Sahib Jai Chand Luthra for the keen interest throughout the course of this investigation. Thanks are also due to the Agricultural Chemist for the help rendered in carrying out the chemical analysis of the cotton roots.

X. Summary

1. A synthetic nutrient medium equivalent to the composition of cotton roots has been described.

2. The dependence of growth upon the most important constituents of the nutrient medium has been studied.

3. Both the *Rhizoctonia* spp. are fairly fast-growing fungi with optimum growth at 30°C. and have a very wide range of toleration to acidity and alkalinity, that is between pH 2·4 and 9·2.

4. Carbon dioxide has a depressing effect on the growth of *Rhizoctonia bataticola* and *Rhizoctonia solani*.

5. Upper thermal death points of *Rhizoctonia bataticola* and *Rhizoctonia solani* are very high, being 68°C. and 60°C. respectively.

6. The parasitic activity of *Rhizoctonia solani* is increased in the presence of certain other fungi in the inoculum.

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ON SOME OF THE FACTORS THAT INFLUENCE THE ERROR OF FIELD EXPERIMENTS WITH SPECIAL REFERENCE TO SUGARCANE

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Introduction

The successful interpretation of field experiments of modern days depends to a large extent on the correct estimation of the error due to chance. But agricultural experiments are subject to errors due to a multiplicity of causes, and most of them are beyond control. The heterogeneity of the soil, the conditions of temperature, humidity and rainfall, etc., that vary from year to year are factors beyond man's control. The crops on the border have more sunshine, more air and more space for growth than those in the interior which have to struggle among themselves for a requisite supply of these natural factors for their full development and growth. The natural result is that the border crops are usually better than the interior and this high yield of the border region increases the mean and the variance. In manurial experiments there is always a certain amount of admixture of treatments between adjoining plots by leaching. Analyses of results of experiments in Europe and America, specially designed, have proved conclusively that size and shape of plots exercise a good deal of influence on the error of an experiment.

Considering the various factors that come into play in the conduct of agricultural experiments in the field, one cannot help concluding that field experiments can never be performed under ideal conditions. Under the circumstances, the best thing that can be done is to eliminate as far as possible the error that may be introduced due to known causes. In designing the plan of a field experiment, particular attention may be paid to statistical requirements so as to reduce the errors to a practical minimum.

Another important factor which influences any estimate of error is the number of replications. The accuracy of the variance and the mean depends on the number of replications used. It has been found that the precision rapidly increases as the replications are increased. But after a certain stage, the increase

in the accuracy is not commensurate with the labour and money spent on the increase in replications. Hence the minimum number of replications required to satisfy the demands of statistics may be determined.

Not much work has been done in India as to the best size of plots suited for experiments on sugarcane. In this paper it is proposed to determine a suitable size of plots and the number of replications required so that the variance may be reduced to a practical minimum for experiments on cane.

Review of literature

It would be difficult indeed to review within the compass of a short paper like this, the vast amount of literature that has steadily accumulated during the last two decades on the size of plots for agricultural experiments, not necessarily sugarcane. We are content to make only a passing reference to some of the more important contributions made by agricultural workers in Europe, America and India on the subject and the broad conclusions arrived at by them.

Mercer and Hall [1911] were the pioneer workers in the field, and they find that in field trials, however uniform and careful the management may be, there will be an experimental error attached to the result due to so many incalculable factors that may be described as casual. The error diminishes with the size of the plot, but the reduction is small when the area of the plot is above 1/40 acre. The error can be diminished by increasing the number of replications, but there is not much to be gained by increasing the number above five. Day [1920] concludes his paper on the relation of size, shape and number of plots to probable error to field experimentation with the remark that increasing the size of plot to 1/20 acre and probably much beyond that reduces variation. More accurate results are obtained by long and narrow plots extending in the direction of greatest variation. An increase in the number of replications increases the accuracy of the result. Variation is reduced when the size of plots is increased keeping the number of replications constant. Wood and Stratton [1910] remark that the probable error does not depend on the size of the plot. It is not larger for smaller plots of 1/40 or 1/80 acre. In large plots, the errors due to individual variation and inaccuracies of weighing are reduced. But this reduction is balanced by systematic errors due to variation of soil which is greater for bigger plots. Christidis [1931] in a general discussion on the effect of shape of plots in the reduction of error in field experiments finds that a square plot is no way superior to long and narrow ones. According to him there are some practical considerations which may make it desirable to increase the width of the plot in relation to its length. Thompson [1934] in his study on the influence of plot size, shape, orientation and the number of replications on the experimental error on sweet-potatoes draws the following conclusions :—Variations among plots increase as the plot approaches a square. It is not advisable to suggest that any one plot size would be the most desirable.

Equally satisfactory results may be obtained from plots of different sizes provided they are replicated a sufficient number of times. Immer [1932] in his investigations on size and shape of plots in relation to field experiments with sugar beet notices that standard error decreases in general with increased size of plot. Efficiency in use of land also decreases as the size of the plot increases. Sayer and Vaidyanathan [1936] recently found with reference to unirrigated sugarcane that an experimental plot-size between 12/242 acre and 16/242 acre is desirable, that the ratio of length to width of the experimental plot should be at least 6·7 and that the minimum number of replications required is four.

Material

In continuation of the previous years' experiments with Co. 205 and Co. 213, the results of which will be published shortly, under the joint authorship of Wynne Sayer and Rao Bahadur M. Vaidyanathan, another experiment was laid out with Co. 210 during the year 1933-34. The choice for this particular variety was made for the reason that Co. 210 like Co. 213 is recognised as the standard variety of cane of North Bihar and grown under unirrigated condition. All cane experiments, manurial and cultural, are laid down mostly under this variety or Co. 213.

The present experiment was laid out at the New Area, Pusa 'A' Block, on an area of six acres. This land is solely devoted to sugarcane experiments and forms a separate unit for this purpose. The land is of light nature and represents the average soil of the district on which cane is grown for the sugar industry. It is farmed on a regular system of crop rotation that has been found suitable for cane growing on the type of soil represented in this area. The previous cropping history and the rotation are given below :—

Year	*Kharif*	*Rabi*
1929-30	Cane	Cane
1930-31	Pulses	Oats
1931-32	Maize	Berseem
1932-33	Pulses	Fallow
1933-34	Cane	Cane

The experiment was laid out in six blocks, each separated by a three-feet bund. The cane was planted eye to eye in rows three feet apart with six feet of the ends doubled on the 20th February, 1933. The standard manure (40 lb. nitrogen as rape-cake and 50 lb. P_2O_5 as superphosphate) which forms the usual dressing for all cane crop on the Farm was applied at the time of planting. The

operation of earthing up the rows was completed just before the break of monsoon. The record of rainfall during the year of the experiment is given below :—

February, 1933	0·26	inches
March „	*Nil*	
April „	1.38	„
May „	4·09	„
June „	6·75	„
July „	21·69	„
August „	11·35	„
September „	5·45	„
October „	1·89	„
November „	*Nil*	
December „	*Nil*	
January 1934	0·59	„
Total	53·43	inches

Average rainfall for twenty years=47·00 inches.

At harvest, the crop was fairly uniform in growth and there was very little lodging.

The cutting of canes was started on the 3rd January, 1934 and it took twenty-four days to finish the entire work. It would have been possible to complete the work seven days earlier, but the earthquake of the 15th January caused interruption and the work was consequently prolonged.

Before harvesting for final yields, the border crop with a minimum width of six feet was removed from all sides of the six blocks. As a result of this it will be seen in the Appendix, an alley was formed between the adjacent blocks. Each block measured 240 feet in length and was divided into four equal sections. The ultimate plot thus obtained consisted of one row a piece and measured 60 ft. × 3 ft. or 1/242 acre. The canes from these plots were cut, stripped and weighed separately guarding against all possible sources of error. The area thus harvested after excluding border crop was four acres and consisted of 121 rows of 480 ft. in length excluding the alley between the adjacent plots. The number of yield figures recorded was 968.

Methods employed for the statistical analysis

Plot size.—Tables giving the yields for various plot sizes, *viz.*, 60 ft.×3 ft., 120 ft.×3 ft., 60 ft.×6 ft.....up to 24/242 acre, were drawn up and the coefficient of variation after eliminating the soil variability was calculated for the respective plot sizes. In getting the yield for different areas, care was taken to see that the figures from one block were not combined with any of the other blocks. In adopting such a procedure, a few observations from each block for certain combinations had to be omitted.

The standard error was calculated after eliminating the error due to rows and columns. The method adopted will be clear from the tables of analysis of variance given below for plot sizes 60 ft.×3 ft. and 240 ft.×9 ft.

TABLE I

Analysis of variance (60 ft.×3 ft.)

Variance due to	Degrees of freedom	Sum of squares	Mean square	Standard deviation
Rows . . .	120	566111·7356	..	..
Columns . . .	7	210908·2843	..	..
Residual . .	840	954366·4344	1136·1505	33·71
Total .	967	1731386·4543	1790·4720	42·31

TABLE II

Analysis of variance (240 ft.×9 ft.)

Variance due to	Degrees of freedom	Sum of squares	Mean square	Standard deviation
Rows .	38	5592808·3490	..	..
Columns .	1	1458097·1599	..	..
Residual .	38	4263415·2151	112195·1372	334·96
Total .	77	11314320·7240	146939·2300	383·33

Table III gives for each combination the size of plots, the number of rows and columns, the mean, the standard deviation and the coefficient of variation after eliminating soil variability. In columns 10 and 11 are given the standard deviation and the coefficient of variation without any elimination. This will enable us to judge the extent to which the error due to soil heterogeneity has been eliminated. The approximate number of replications necessary, so that the difference between the mean of the sample and the population to which the sample belongs is five per cent or ten per cent of the population mean, is also given in the last two columns of the table for various plot sizes. The methods adopted for determining these have been indicated briefly in the course of the discussion of the results.

TABLE III

Results of analysis of data for plot size and shape

Serial No.	Plot size	Area (acre)	Ratio of length to width	No. of rows	No. of columns	Mean	After elimination of soil variation in two directions		Without any elimination		Number of replications Odds 1 : 30	
							S. D.	C. V.	S. D.	C. V.	5 per cent	10 per cent
1	2	3	4	5	6	7	8	9	10	11	12	13
1	60 ft.× 3 ft.	1/242	20	121	8	270·8290±1·0835	33·71±0·7662	12·4470±0·2873	42·31	15·6224	25	9
2	60 ft.× 6 ft.	2/242	10	60	8	541·4021±2·8162	61·70±1·9914	11·3964±0·3726	79·37	14·6601	22	8
	120 ft.× 3 ft.	2/242	40	121	4	541·6581±2·6832	59·03±1·8973	10·8980±0·3544	75·05	13·8556	21	7
3	60 ft.× 9 ft.	3/242	6·7	39	8	810·0144±4·9769	87·91±3·5192	10·8529±0·4754	114·45	14·1294	21	7
	180 ft.× 3 ft.	3/242	60	121	2	813·5165±5·1130	79·54±3·6154	9·7773±0·4487	102·60	12·6119	17	7
4	60 ft.×12 ft.	4/242	5	29	8	1083·5388±7·7182	117·56±5·4576	10·8496±0·551	153·60	14·1758	21	7
	120 ft.× 6 ft.	4/242	20	60	4	1082·8042±7·1631	110·97±5·0651	10·2484±0·5136	143·12	13·2175	18	7
	240 ft.× 3 ft.	4/242	80	121	2	1083·3161±7·3571	114·45±5·2023	10·5649±0·5263	134·00	12·3695	20	7
5	60 ft.×15 ft.	5/242	4	22	8	1347·5398±10·8996	144·60±7·7072	10·7307±0·6263	184·88	13·7198	20	7
6	60 ft.×18 ft.	6/242	3·3	19	8	1624·0987±13·9291	171·73±9·8493	10·5739±0·6646	222·46	13·6974	20	7
	120 ft.× 9 ft.	6/242	13·3	39	4	1620·0288±13·1713	164·51±9·3135	10·1548±0·6315	210·57	12·9979	18	7
	180 ft.× 6 ft.	6/242	30	60	2	1626·4333±13·9012	152·28±9·8296	9·3628±0·6097	197·16	12·1222	16	6
7	60 ft.×21 ft.	7/242	2·9	16	8	1895·6484±17·1659	194·21±12·1381	10·2450±0·7030	254·81	13·4418	18	7
8	60 ft.×24 ft.	8/242	2·5	14	8	2153·0938±21·5100	227·64±15·2099	10·5724±0·7741	291·19	13·5243	20	7
	120 ft.×12 ft.	8/242	10	29	4	2167·0776±19·8118	213·38±14·0091	9·8464±0·6527	279·29	12·8879	17	7
	240 ft.× 6 ft.	8/242	40	60	2	2165·6083±20·2247	221·55±14·3010	10·2304±0·7251	258·79	11·9500	18	7
9	60 ft.×27 ft.	9/242	2·2	11	8	2397·7216±25·6513	240·63±18·1382	10·0358±0·7641	303·05	12·6391	17	7
	180 ft.× 9 ft.	9/242	20	39	2	2432·5128±26·2564	231·89±18·5661	9·5329±0·7702	291·41	11·9798	16	7
10	60 ft.×30 ft.	10/242	2	11	8	2695·0795±29·4388	276·16±20·8164	10·2468±0·7805	353·72	13·1247	18	7
	120 ft.×15 ft.	10/242	8	22	4	2695·0795±28·5071	267·42±20·1576	9·9225±0·7553	337·85	12·5358	17	7

11	60 ft. × 33 ft.	11/242	1·8	10	8	2956·6813±34·0117	304·21±24·0499	10·2889±0·8220	394·35	13·3376	18	7
12	60 ft. × 36 ft.	12/242	1·7	8	8	3200·4141±41·4225	331·38±29·2901	10·3543±0·9250	400·39	12·5106	19	7
	120 ft. × 18 ft.	12/242	6·7	19	4	3248·1974±36·5184	318·36±25·8224	9·8011±0·8026	408·51	12·5765	17	7
	180 ft. × 12 ft.	12/242	15	29	2	3257·1293±39·7031	302·37±28·0743	9·2833±0·8694	388·43	11·9255	15	6
	240 ft. × 9 ft.	12/242	26·7	39	2	3240·0577±37·9268	334·96±26·8183	10·3381±0·8366	383·33	11·8310	19	7
13	60 ft. × 39 ft.	13/242	1·5	8	8	3497·1406±43·0013	344·01±30·4065	9·8369±0·8779	441·00	12·6103	17	7
14	60 ft. × 42 ft.	14/242	1·4	8	8	3791·2969±47·0963	376·77±33·3021	9·9378±0·8870	487·09	12·8476	17	7
	120 ft. × 21 ft.	14/242	6	16	4	3791·2969±45·1250	361·00±31·9082	9·5218±0·8493	470·12	12·4000	16	7
15	60 ft. × 45 ft.	15/242	1·3	6	8	3939·1146±61·7289	427·67±43·6489	10·8570±1·1211	469·09	11·9085	21	7
	180 ft. × 15 ft.	15/242	12	22	2	4047·4886±57·9324	384·28±40 9644	9·4943±1·0213	466·66	11·5298	16	7
16	60 ft. × 48 ft.	16/242	1·3	6	8	4218·7604±65·4788	453·65±46·8005	10·7532±1·1102	512·82	12·1567	20	7
	120 ft. × 24 ft.	16/242	5	14	4	4306·1875±56·7716	424·84±40·1436	9·8658±0·9413	535·55	12·4366	17	7
	240 ft. × 12 ft.	16/242	20	29	2	4334·1552±58·0322	441·96±41·0350	10·1972±0·9566	512·45	11·8235	18	7
17	60 ft. × 51 ft.	17/242	1·2	6	8	4497·9281±66·9149	463·60±47·3160	10·3070±1·0631	543·07	12·0738	18	7
18	120 ft. × 27 ft.	18/242	4·4	11	4	4795·4432±69·5150	461·11±49·1545	9·6154±1·0345	562·23	11·7243	16	7
	180 ft. × 18 ft.	18/242	10	19	2	4877·0395±73·4036	452·49±51·9042	9·2780±1·0734	565·80	11·6013	15	6
19	120 ft. × 30 ft.	20/242	4	11	4	5390·1591±79·8704	529·80±56·4769	9·8290±1·0579	660·04	12·2453	17	7
	240 ft. × 15 ft.	20/242	16	22	2	5390·1591±84·9267	563·34±60·0522	10·4518±1·1263	621·15	11·5238	19	7
20	120 ft. × 33 ft.	22/242	3·6	10	4	5913·3626±91·7804	580·47±64·8985	9·8163±1·1051	734·48	12·4207	17	7
21	120 ft. × 36 ft.	24/242	3·3	8	4	6400·8281±110·4676	624·90±78·1125	9·7628±1·2320	755·30	11·8000	17	7
	240 ft. × 18 ft.	24/242	13·3	19	2	6496·3947±107·8091	664·58±76·2325	10·2300±1·1857	752·96	11·5904	18	7

Discussion of the results

Plot size and shape.—The relation between the area and the coefficient of variation after eliminating soil heterogeneity (the effect of shape being neglected) is clearly indicated in the table given below :—

Table IV

Relation between the area of plots and coefficient of variation

Serial No.	Area (acre)	Frequency	Average C. V.
1	1/242	1	12·4470
2	2/242	2	11·1472
3	3/242	2	10·3151
4	4/242	3	10·5543
5	5/242	1	10·7307
6	6/242	3	10·0305
7	7/242	1	10·2450
8	8/242	3	10·2164
9	9/242	2	9·7844
10	10/242	2	10·0847
11	11/242	1	10·2889
12	12/242	4	9·9442
13	13/242	1	9·8369
14	14/242	2	9·7298
15	15/242	2	10·1757
16	16/242	3	10·2721
17	17/242	1	10·3070
18	18/242	2	9·4467
19	20/242	2	10·1402
20	22/242	1	9·8163
21	24/242	2	9·9964

It is seen that the variation, with the exception of one or two cases, steadily decreases as the area is increased from 1/242 to 9/242 acre. Commencing with the value 12·4470, the coefficient of variation comes down to 9·7844, which is significantly lower than the starting value. For areas greater than 9/242 acre, the percentage variation fluctuates between 9·4467 and 10·3070 ; there is no significant difference between any two values. Hence it can be concluded that any convenient area beyond 9/242 acre can be taken as the best size of plots suited for sugarcane.

Table V shows the manner in which the coefficient of variation is effected by changing the shape of plots (no consideration being made of the area).

TABLE V

Relation between the shape of plots and coefficient of variation

Ratio of length/width considered	No. of plot sizes considered	Average C. V. (for the several plot sizes)
Between 1 and 2	8	10·3227
Between 2 and 3	3	10·2844
Between 3 and 5	8	10·1304
Between 5 and 10	7	10·0884
Between 10 and 20	9	10·2266
Between 20 and 30	2	9·8505
Between 30 and 80	4	10·3677

There seems to be no difference between the coefficient of variation for the various plot-sizes. But, broadly speaking, the coefficient of variation diminishes until the ratio of length to width is thirty, and beyond this limit it increases. It may be noted that the percentage variation for the class interval 10—20 is 10·2266 which is higher than the preceding and succeeding values. This is due to the inclusion of the coefficient of variation for the area 1/242 acre which is an abnormally high figure. Thus, it can be seen that it is not advisable to increase the ratio of length to breadth of experimental plots above thirty.

The conclusions drawn above are to be taken with a little reserve, for we have not so far taken into account the variation of the coefficient of variation when both the area and the shape of plot change simultaneously. This can be done by making use of Table III. A careful scrutiny of the table indicates that the percentage variation diminishes as the ratio of length to breadth is increased up to a certain limit, keeping the area constant. Similarly when the shape is fixed, the variation can be diminished by increasing the area.

To fix the best size of plots suited for experiments, it is necessary to take into account both the theoretical and the practical aspects. In practice, it is not advisable to have either very small plots or plots of considerable size to secure efficient cultivation and other agricultural operations. The most feasible thing seems to be to strike a golden mean. Any area lying between 9/242 and 15/242 acre, having the shape so adjusted that the ratio of length to breadth lies anywhere between five and fifteen, can be taken as the best size and shape of plots for experimental purposes on canes.

REPLICATIONS

A satisfactory way of determining the number of replications necessary is by using the Student's distribution of Z, (where Z is equal to the deviation from population mean/the standard deviation of the sample), published in "Tables for Statisticians and Biometricians, Part I," page 36. If the deviation, the

standard deviation of the sample and the probability are known, then n can be read directly from the above table. Assuming the difference between the population and sample means to be five per cent or ten per cent of the sample mean, then n for odds 30 to 1 (*i.e.*, for the probability of 0·97 of not exceeding the deviation) can be read from the table. Thus for mean=270·8, standard deviation=33·71, and deviation=ten per cent, n works out to 9.

Summary and conclusions

1. The yields of Co. 210 from 968 plots of 1/242 acre, each 60 ft. × 3 ft., have been analysed with a view to determine the following :—

(*i*) the best size and shape of plots for experiments on canes, (*ii*) the number of replications required to get satisfactory results.

2. The analysis shows that the error of field experiments is partly governed by size and shape of plots. The percentage variation can be diminished by increasing the plot size up to 9/242 or 1/27 acre. Nothing is gained from theoretical aspects by increasing the area beyond 1/27 acre. But for the sake of convenience we can have any area between 1/27 and 15/242 or 1/16 acre. As regards the shape of plots, the investigations indicate that the error can be diminished to a slight extent by increasing the ratio of length to breadth approximately up to a limit of thirty. But in practice it is convenient to adjust the size of plots in such a way as to have the length five to fifteen times the breadth.

3. The number of replications required depends on the percentage variation. If the coefficient of variation is small, it is necessary to have only a few number of repetitions. But if the error is large, it is advisable to repeat the experiment a greater number of times. For land of average variability, six to nine replications will serve the purpose.

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Appendix

Tonnage experiment with Co. 210, 1934. New Area, Block—Pusa A.

(Each row is 3 feet apart and the cuts are 60 feet. Area of each plot is 180 square feet; yields in pounds.)

Row No.	Block I				Block II			
	A	B	C	D	E	F	G	H
1	280·5	281·0	313·0	290·0	290·0	264·0	202·0	260·0
2	282·0	286·0	332·5	265·0	282·5	251·0	232·0	243·0
3	251·0	255·0	305·0	299·0	234·5	230·0	224·0	243·0
4	301·0	288·0	286·0	252·5	243·0	207·0	211·5	211·0
5	288·0	255·5	303·0	277·0	226·5	225·0	218·5	243·0
6	310·0	273·5	259·0	248·0	212·0	207·0	216·0	216·0
7	263·5	251·0	272·5	273·0	240·0	261·0	233·0	230·0
8	272·5	284·0	294·5	310·0	261·0	235·0	231·0	233·5
9	296·0	266·5	291·0	301·0	319·0	247·5	232·5	243·5
10	264·5	323·5	271·0	285·0	275·0	214·0	193·0	252·0
11	281·0	281·0	286·0	298·0	276·0	258·0	234·5	265·0
12	320·0	283·0	251·0	261·0	241·5	220·5	211·0	222·5
13	271·0	308·5	272·0	299·0	283·0	239·5	222·0	285·0
14	281·0	292·0	248·5	315·0	230·0	229·0	181·0	215·5
15	284·0	263·0	275·0	310·0	278·5	208·0	204·0	227·0
16	301·0	281·5	270·0	330·0	250·0	249·0	215·5	274·0
17	294·5	278·0	280·5	319·0	254·0	208·0	206·5	208·0
18	311·5	298·5	319·0	306·0	271·0	259·0	202·5	215·5
19	325·0	267·0	288·0	314·0	246·5	226·0	191·5	222·0
20	310·0	283·0	283·5	318·0	268·0	224·0	202·0	233·5
21	297·0	296·0	285·5	301·0	280·0	242·0	214·0	211·0
22	301·0	274·0	291·5	303·5	270·0	257·5	262·0	246·0
23	335·0	272·0	302·0	290·0	260·5	218·5	195·0	200·5
24	314·5	293·0	292·0	318·5	259·5	239·0	215·5	249·5
25	341·5	278·0	307·0	277·0	244·5	207·0	208·0	274·0
26	305·0	297·0	304·0	302·5	265·5	224·0	188·5	280·0
27	337·0	315·0	317·0	294·0	253·0	231·5	223·5	283·0
28	338·0	293·0	289·0	271·5	239·0	188·5	197·5	250·0
29	311·0	306·0	325·0	278·0	252·0	232·0	204·5	262·0
30	270·0	338·0	338·0	261·0	199·5	213·5	197·5	226·0
31	280·0	324·0	311·0	279·0	243·0	221·5	214·0	278·0
32	308·0	306·0	363·0	261·0	270·0	231·0	217·0	273·0
33	304·0	299·0	331·0	280·0	249·5	253·5	248·0	294·0
34	315·0	303·0	306·0	304·0	246·5	264·0	235·0	260·0
35	315·5	339·0	335·5	311·0	275·0	305·0	268·0	283·0
36	342·5	356·0	316·5	296·5	269·0	279·0	283·0	269·0
37	293·0	335·0	343·0	304·0	256·5	270·0	295·0	292·0
38	303·0	325·0	354·0	289·0	290·5	275·0	263·0	287·0
39	307·0	325·0	338·5	305·0	285·5	277·5	299·0	337·0
40	342·0	340·0	317·0	306·0	265·0	269·5	269·5	293·0

APPENDIX—*contd.*

Row No.	Block I				Block II			
	A	B	C	D	E	F	G	H
41	320·0	305·0	316·0	253·5	293·0	265·5	280·0	329·0
42	282·0	255·0	262·0	277·0	224·5	295·0	280·0	262·0
43	304·0	311·0	314·5	279·0	254·5	291·0	249·0	286·5
	Block III				Block IV			
44	326·0	329·0	333·0	306·5	266·0	275·0	265·0	257·0
45	269·0	257·0	295·5	312·0	273·0	246·0	248·0	266·0
46	255·0	300·0	325·0	256·0	244·5	265·0	217·0	244·5
47	288·0	287·0	261·0	244·0	255·5	225·5	206·5	248·0
48	284·5	281·0	272·0	258·5	244·0	247·5	224·5	299·0
49	304·5	308·0	303·5	263·0	259·5	281·0	247·0	280·0
50	295·5	286·0	325·0	267.0	254·0	253·5	238·0	260·0
51	303·0	292·0	295·0	231·5	277·5	268·0	235·0	272·0
52	304·0	258·0	316·0	225·0	261·5	270·5	238·0	283·5
53	264·0	302·0	273·0	228·0	250·0	252·0	222·0	295·0
54	309·0	274·5	306·5	235·0	242·0	255·0	225·0	256·0
55	297·0	303·0	328·5	276·0	232·0	257·0	266·5	248·0
56	296·0	294·5	329·5	237·5	225·5	238·0	242·0	240·0
57	319·0	295·5	311·0	256·0	234·0	249·0	261·0	241·0
58	310·0	270·0	325·0	262·5	245·5	252·5	238·5	260·0
59	294·0	310·0	283·0	283·5	236·5	239·0	241·0	231·0
60	295·0	278·0	280·0	300·0	267·0	254·5	242·0	241·0
61	296·0	304·5	329·5	294·5	250·0	233·0	221·0	239·5
62	293·0	315·0	288·5	316·0	271·0	241·0	237·5	292·0
63	292·0	296·0	276·0	313·0	245·0	300·0	267·5	300·5
64	347·0	312·5	276·5	285·0	230·0	289·0	255·5	264·0
65	262·0	298·5	262·0	300·0	255·0	275·5	256·0	237·0
66	323·5	354·5	266·5	309·5	289·0	299·0	246·0	277·0
67	291·0	279·0	287·0	253·0	281·5	198·0	214·0	281·0
68	306·5	294·0	324·5	344·0	291·0	240·0	255·5	267·0
69	353·0	298·0	287 0	365·5	304·5	267·0	276·0	289·0
70	280·0	262·5	278·0	378·5	339·5	278·0	262·5	335·0
71	296·0	294·0	304·0	316·5	299·5	212·0	265·0	276·0
72	302·0	333·0	269·5	354·0	328·0	303·0	247·5	280·0
73	314·0	332·5	284·0	356·5	311·0	286·5	227·5	269·0
74	336·0	404·5	350·5	350·0	306·5	307·5	226·5	251·0
75	320·0	371·5	334·0	383·0	325·5	285·0	256·5	256·0
76	296·0	333·0	381·5	351·0	272·0	291·0	260·5	227·0
77	324·5	311·0	355·5	316·5	296·0	328·0	259·0	312·5
78	302·5	336·0	284·0	279·0	298·0	327·0	243·0	241·0
79	314·5	317·5	261·0	323·0	296·5	289·5	239·0	222·0
80	290·0	366·5	262·0	359·0	330·0	365·0	257·0	249·0
81	360·0	313·5	310·5	380·0	262·5	360·5	253·5	228·0
82	352·0	371·0	304·0	380·0	310·0	387·0	277·0	254·0
83	345·0	342·5	309·0	347·0	341·5	354·0	288·5	260·5
84	347·0	404·0	263·0	299·0	296·0	291·5	292·0	287·0
85	327·0	367·0	292·0	376·0	357·5	405·0	288·5	304·0
86	286·5	361·0	332·0	369·0	327·0	348·0	308·0	317·5
87	293·0	320·0	306·5	299·5	332·5	294·0	269·0	274·5

APPENDIX *—concld.*

Row No.	Block V				Block VI			
	A	B	C	D	E	F	G	H
88	250·0	277·0	308·5	241·0	247·5	303·5	233·0	286·0
89	221·0	232·5	277·5	317·5	296·0	327·5	279·0	297·5
90	185·0	245·0	306·0	280·0	278·0	279·5	247·0	282·0
91	155·5	205·0	243·0	261·0	240·0	307·0	227·0	274·5
92	250·5	200·0	243·5	271·0	269·5	274·0	218·0	291·0
93	205·0	220·5	276·5	282·5	286·5	290·0	226·0	261·5
94	257·0	232·5	275·5	255·5	291·5	297·0	247·0	297·5
95	211·0	210·5	267·0	279·0	289·5	282·5	258·5	254·0
96	176·0	186·0	262·0	285·0	273·5	278·5	234·5	255·0
97	175·5	183·0	220·5	272·0	257·0	255·5	226·0	225·0
98	214·5	188·0	239·0	254·5	273·0	235·5	227·0	248·5
99	186·0	160·0	214·0	269·0	263·0	269·5	213·0	268·5
100	188·5	164·0	206·5	251·5	250·0	268·0	258·0	262·0
101	181·5	165·5	223·5	301·0	230·0	282·5	245·0	235·0
102	191·0	186·0	207·0	259·0	257·0	292·5	262·5	208·0
103	191·5	182·0	221·0	230·0	230·0	273·0	256·5	243·0
104	188·5	189·5	211·5	249·0	266·5	276·0	243·0	301·5
105	194·5	204·0	201·0	234·0	227·0	281·0	224·0	287·0
106	233·5	222·5	191·0	236·0	235·0	262·5	234·5	295·0
107	203·5	210·0	219·0	236·5	266·0	264·0	271·0	298·5
108	232·5	236·0	186·0	210·5	235·0	300·0	225·0	266·0
109	203·5	216·5	215·0	228·5	266·5	312·0	251·0	322·5
110	202·0	222·0	206·0	255·5	227·5	324·5	212·5	247·0
111	206·0	198·5	189·0	258·5	258·5	275·5	216·0	264·5
112	192·5	219·0	218·0	343·0	241·0	283·5	249·5	260·0
113	226·5	270·5	231·0	327·5	252·0	283·0	277·0	285·0
114	174·5	210·0	246·5	343·0	289·5	276·0	204·0	270·5
115	201·5	212·0	195·0	310·5	361·0	313·0	231·5	301·5
116	216·0	262·5	225·5	274·0	407·0	303·5	208·5	234·5
117	182·5	231·5	227·5	312·0	315·0	322·0	219·0	279·0
118	212·5	259·5	219·0	244·0	317·0	273·0	223·5	265·5
119	204·0	255·0	245·0	272·5	266·5	211·0	240·0	271·5
120	244·0	222·5	239·0	259·5	211·5	212·5	237·5	255·5
121	222·0	255·5	271·5	311·0	243·0	244·0	244·5	319·0

THE CLASSIFICATION OF THE AUTUMN RICES OF THE PUNJAB AND WESTERN UNITED PROVINCES

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I. Introduction

The total amount of annual precipitation and its distribution largely determines the extent to which rice is cultivated in India. Wherever the rainfall is abundant and fairly uniform in distribution, rice is a crop of primary importance, but in areas such as the Punjab and the Western United Provinces where the monsoon is less bountiful and reliable, rice cultivation is relatively unimportant. Of the total paddy area in British India, Punjab and the Western United Provinces together claim a little over two per cent. The distribution of this is given in Table I.

TABLE I

Area under rice in the districts of the Punjab, 1933-34

District	Area
Hissar	7,775
Rohtak	455
Gurgaon	18
Karnal *	66,350
Ambala *	57,684
Simla	1,051
Kangra *	116,500
Hoshiarpore *	35,024
Jullundur	2,091
Ludhiana	1,281
Ferozepore	24,186
Lahore	31,978
Amritsar	34,768
Gurdaspur *	75,506
Gujranwala *	164,758
Sheikhupura *	142,794
Gujrat	20,235
Shahpur	5,866
Jhelum	1,134
Rawalpindi	995
Attock	14
Mianwali	1
Montgomery	28,109
Lyallpur	2,506
Jhung	2,270
Multan	32,897
Muzaffargarh	37,188
Dera Ghazi Khan *	69,960
Sialkot *	71,392

Area under rice in some districts of the United Provinces, 1933-34

District	Area
Dehra Dun	24,898
Saharanpore *	106,594
Muzaffarnagar *	36,610
Meerut	17,830
Bulandshahr	2,230
Bareilly *	161,235
Bijnor *	153,689
Badaun	39,378
Moradabad *	119,154
Pilibhit *	121,426
Aligarh	1,625
Muttra	10
Agra	25
Mainpuri	26,130
Etah	11,377

It is only in the districts marked * that the cultivation of rice is somewhat important. The staple food of the people in these and other districts in the area under consideration, as a whole, is wheat, and rice forms a regular article of diet only in the case of upper classes.

The climatic conditions prevailing in these parts do not favour the successful growth of winter or long duration paddy varieties. In contrast, therefore, to the case of Bengal and Bihar, early maturing types are almost invariably grown, the crop being harvested before the onset of cold weather, *i.e.*, about the end of October, and the land then sown with a *Rabi* crop.

It will thus be seen that the rice types evolved at Karnal are very different from those classified at Pusa. It is in this respect that the paddy classification described in these pages, differs from the original one. Although the basis of the classification evolved by the senior author remains the same and has been adhered to in entirety, the inclusion of autumn paddy types forms an important supplementary work. Besides, it proves the applicability of the Pusa scheme of classification of rices, to a group of paddy types which are quite distinct from those worked on at Pusa.

II. Material and method

One hundred and fifty-seven paddy samples were collected from the important rice-growing districts of the Punjab and the Western United Provinces. The types isolated in the United Provinces by Sethi [1931] were also kindly supplied by him, but with the exception of a few samples from Dehra Dun, these proved to be late and unsuitable for Karnal conditions.

After eliminating duplicates, forty-one distinct types were isolated from the original collection of 157 kinds. Since the present work is a continuation of that commenced by the senior author at Pusa where 123 strains were isolated and described [Kashi Ram and Chetty, 1934], the types described in the present paper are numbered from 124 to 164. The localities whence the latter were obtained are enumerated in Table II.

Table II

Types isolated from several districts of the Punjab and the United Provinces

Districts	Types isolated
The Punjab—	
Ambala	143, 145, 155
Ferozepore	139, 151
Gujrat	136, 141, 159

TABLE II—*contd.*

Districts	Types isolated
Hissar	128, 133, 157, 158, 161
Karnal	124, 126, 129, 130, 132, 134, 144, 147, 148, 150, 152, 153, 154, 162, 164
Muzaffargarh . . .	142
Sheikhupura	125, 127, 131, 135, 137, 138, 140, 146, 156, 160, 163
United Provinces—	
Dehra Dun	149

III. LITERATURE

The multiplicity of rice varieties and the high degree of physiological adaptation displayed by the paddy plant are probably responsible for the lack of uniformity in methods of classification. Kikkawa [1912], Hector [1916, 1922, 1929], Graham [1913], Thadani and Datta [1928], Sethi and Saxena [1930], etc., have all made contributions to the classification of rices ; these will not be discussed here as they have been recently reviewed [Kashi Ram and Chetty, 1934]. It may be said, however, that two distinct kinds of classificatory schemes have emerged ; those based on morphological characters and those based on both morphological and agricultural characters. Thus Koernicke [1885] used the character of the grain for separating the glutinous rices from the common starchy ones. Kikkawa [1912] employed both morphological and agricultural characters for sorting out the different varieties. On the other hand, Hector [1930] pointed out that the so-called "*Aus*—High Land rice, *Aus*—Low Land rice, *Aman*—transplanted rice and *Aman*—Low Land rice" are difficult to distinguish from each other morphologically, and yet are clearly different as regards their requirements of water, etc. But in Graham's opinion [Graham, 1913] the agricultural characters are less constant than the morphological ones and therefore not sufficiently dependable for use as bases of classification. He mentions that while early, medium, and late rices from Jubbulpore matured, on the average, in 113, 120 and 128 days respectively, rices of the same classes from Raipur matured in 124, 127 and 138 days respectively.

In the present paper, the scheme of classification, based on morphological characters, evolved at Pusa [Kashi Ram and Chetty, 1934] has been adhered to.

IV. Bases of classification

The Pusa classification referred to in the last paragraph of Section III is based on morphological characters, such as the constitution of the rice kernel, presence or absence of clustering in spikelets, length of the outer glumes, and the presence or absence of elongated internodes with aerial roots. After these, the colour of the inner glumes was chosen as the most reliable criterion. Furthermore the inner glumes exhibit a wide range of easily distinguished colours in different varieties and hence afford a ready means of discrimination. Thereafter the colour of the apiculus, outer glumes, kernel, and internode was used, and finally the grain size and shape, which are the most constant of the quantitative characters, were used in separating the ultimate classes.

V. Key to the classification

Starchy rice
Single grained
Short outer glumes
Solitary spikelets
Short internode
Inner glume—straw
Apiculus—colourless
Outer glumes—white
Kernel—white
Internode—green

Grain size—IIb	Slender	124 Beardless, stigma—white
	Medium	125 Beardless, stigma—light purple.
IIc	Very slender	126 Bearded, stigma—light purple.
	Medium	127 Beardless, stigma—white.
IIIc	Slender	128 Beardless, scented.
		129 Tipped.
		130 Tipped later than 129.
		131 Beardless, narrow leafed.

Apiculus—brown
Outer glumes—white.
Kernel—white
Internode—green

Grain size—IIb	Bold	132 Tipped.
	Medium	133 Bearded.

Outer gulmes—brown
Kernel—white
Internode—green

Grain size—IIc	Medium	134 Beardless, later than 135.
IIIc	Medium	135 Tipped.
		136 Bearded.

Apiculus—purple (restricted)

Outer glumes—white		
Kernel—white		
Internode—green		
Grain size—IIc	Slender	137 Leaf sheath purple.
Kernel—light red		
Internode—green		
Grain size—IIb	Medium	138 Beardless.
IIIc	Slender	139 Beardless.
Apiculus—purple (spread)		
Outer glumes—purple		
Kernel—white		
Internode—green		
Grain size—IIb	Medium	140 Bearded, stigma white.
IIIc	Medium	141 Bearded, stigma light purple.
	Bold	142 Bearded.
Inner glumes—olive uniform		
Apiculus—brown		
Outer glumes—white		
Kernel—white		
Internode—green		
Grain size—IIc	Slender	143 Beardless, scented.
	Bold	144 Bearded.
IIIc	Slender	145 Beardless.
Kernel—red		
Internode—green		
Grain size—IIb	Medium	146 Tipped.
Apiculus—brown		
Outer glumes—white		
Kernel—white		
Internode—green		
Grain size—IIc	Slender	147 Beardless.
IIIc	Medium	148 Tipped.
Outer glumes—purple		
Kernel—white		
Internode—light gold		
Grain size—IIb	Medium	149 Beardless, dwarf.
Apiculus—purple		
Outer glumes—purple		
Kernel—white		
Internode—green		
Grain size—IIb	Medium	150 Beardless.
	Bold	151 Tipped.

Inner glumes—olive furrowed on green base
Apiculus—colourless
Outer glumes—white
Kernel—white
Internode—green
Grain size—IIb Bold 152 Beardless.
Kernel—red
Internode—green
Grain size—IIc Medium 153 Beardless.
Inner glumes—brown
Apiculus—brown
Outer glumes—brown
Kernel—white
Internode—green
Grain size—IIc Slender 154 Beardless.
Medium 155 Tipped.
156 Bearded.
IIIc Medium 157 Tipped.
Inner glumes—gold
Apiculus—colourless
Outer glumes—white
Kernel—white
Internode—gold
Grain size—IIb Medium 158 Tipped.
Slender 159 Beardless.
Kernel—light red
Internode—gold
Grain size—IIb Medium 160
IIc Slender 161
Inner glumes—dark gold
Apiculus—colourless
Outer glumes—white
Kernel—white
Internode—gold
Grain size—IIb Medium 162 Beardless.
IIc Medium 163 Beardless.
164 Bearded.

VI. Summary

1. Forty-one types have been isolated from a collection of samples from the important rice-growing districts of the tract under consideration. As late varieties were unsuited to the conditions prevailing at Karnal, the work was limited to the autumn rices.

2. The classifications of previous investigators are briefly referred to. The classification of Kashi Ram and Chetty [1934] is followed in this paper.

3. A key to the classification is given.

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INHERITANCE OF CHARACTERS IN SORGHUM—THE GREAT MILLET

IX. DIMPLED GRAINS *

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(With Plate LIII and one text-figure)

In the varietal collections of sorghum at the Millets Breeding Station, Coimbatore, there is a small group represented by a variety called *Sakkara gulige jonna* (Sugar Pill Sorghum) which comes from the district of Bellary in the Madras Presidency. This sorghum has its grains "dimpled". This dimple is in the nature of a depression towards the top of the grain, and is easily visible where it occurs. The sorghum grain is asymmetrical in the disposition of its endosperm, there being a bump on the side away from the embryo. This bump is towards the outermost glume. The dimple when it occurs is on the bumpy side, and always away from the embryo. The dimple can occur in grains having either a floury or corneous endosperm. When the grains are cut there is always a small hollow (varying in its size) immediately behind the dimple.

Plants having this type of grains are not usually vigorous. In the Bellary district, varieties with this type of grain are not grown on an extensive scale, but select cultivators treasure this variety and grow it in small patches to give them the soft and tasty grains that are consumed in the dough stage. But for this careful preservation, these delicate varieties which are probably mutational in origin, would not have survived.

White, yellow and red types were collected from the Bellary tract. By crossing with a brown-grained variety, a dimpled brown type was fixed. Subsequent to this similar dimpled types have been obtained from Bombay and the Central Provinces. This dimpling occurred in both pearly and chalky grains

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[Rangaswami Ayyangar *et al.*, 1934]. Spotted as these peculiar varieties were in an area predominantly *Sorghum cernuum* Host in distribution, the important types partake of *cernuum* characteristics. For the sake of easy identification and future work on this characteristic type of grain, this variety has been designated *S. cernuum* Host var. *areolatus* Rangaswami.

An examination was made of the manifestation of this dimpling on the ear-head in pure varieties. Taking all degrees of dimpling into consideration, dimpling may be said to occur in practically every grain, the maximum number of grains that did not show the dimpling being less than ten per cent. As will be explained later, this ten per cent could be accounted for as resulting partly from crosses occurring in nature and partly owing to the difficulty of spotting minute dimples.

The progress of dimpling was watched in thirty-three ear-heads of six differing varieties. It takes forty to forty-five days for a grain to ripen from the time of pollination. Young grains in the early milky stage begin to manifest dimpling even when they are twelve days old. In another fifteen days dimpling ceases. It is at the stage when dimpling is nearing completion that the grains are most prized for eating.

Crosses (reciprocal) were made between dimpled and normal (non-dimpled) grain types. In all cases where the non-dimpled grain type was used as the mother parent, the crossed grains were also non-dimpled. In the reciprocal crosses with the dimpled grain type as the mother parent, all the crossed grains were non-dimpled.

First generation plants were raised from these crosses. It was noted that the grains in the ear-heads raised, consisted of a mixture of both dimpled and non-dimpled grains. Counts taken in forty-nine hybrid ear-heads are presented in Table I.

TABLE I

Counts of dimpled and non-dimpled grains in hybrid ear-heads from eight artificial crosses

Head No.	No. of grains		Percentage of dimpled grains
	Non-dimpled	Dimpled	
1	2159	716	24·9
2	911	284	24·0
3	1268	401	24·0
4	3037	916	23·2
5	1087	318	23·0
6	1706	510	23·0

TABLE I—*contd.*

Head No.	No. of grains		Percentage of dimpled grains
	Non-dimpled	Dimpled	
7	1934	569	22·7
8	2110	613	22·5
9	2029	582	22·3
10	2683	770	22·3
11	2019	578	22·2
12	1468	416	22·1
13	1623	460	22·1
14	1195	337	22·0
15	1648	464	22·0
16	2085	590	22·0
17	2933	822	21·9
18	2285	636	21·8
19	1716	471	21·5
20	2211	597	21·3
21	2736	733	21·1
22	1579	425	21·0
23	2397	635	20·9
24	1738	458	20·9
25	2392	628	20·8
26	2530	655	20·6
27	2833	732	20·5
28	1617	415	20·4
29	1910	487	20·3
30	2193	553	20·1
31	1464	378	20·0
32	1971	489	19·9
33	2340	573	19·7
34	2081	509	19·7
35	1497	366	19·7
36	1504	366	19·6
37	2240	542	19·5
38	2190	532	19·5
39	2353	572	19·5
40	1332	322	19·5
41	2440	589	19·4
42	1466	351	19·3
43	1638	385	19·0
44	1944	457	19·0
45	2093	488	18·9
46	2931	682	18·9
47	2629	606	18·7
48	2609	597	18·6
49	2956	675	18·6
Total	99710	26250	20·8

FIG. 1. Top : Non-dimpled and Dimpled grains—(Front view).

Bottom : Non-dimpled and Dimpled grains—(Side view, in section).

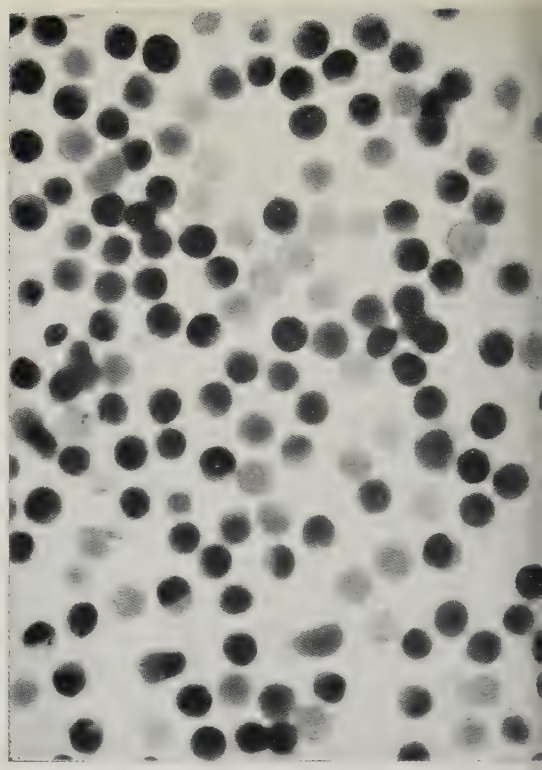

FIG. 2. Pollen dimorphism—Deep and light stain grains from hybrid earhead.

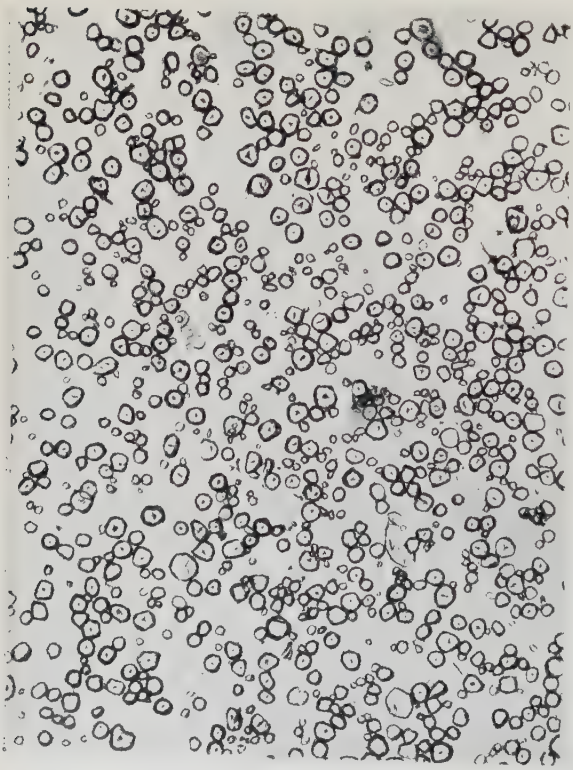

FIG. 3. Starch grains -Non-dimpled seed.

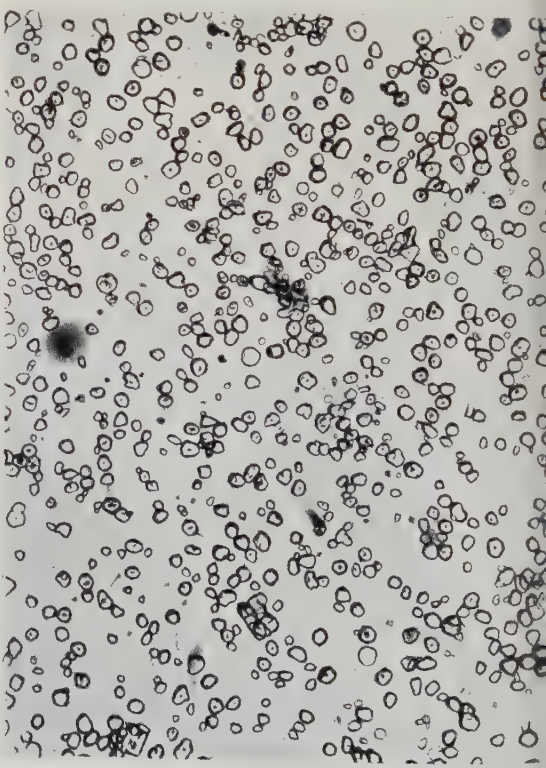

FIG. 4. Starch grains—Dimpled seed.

The above numbers give an approximate 3 : 1 ratio, the non-dimpled grains being in excess. A practical difficulty is experienced in the sorting of the grains which might possibly explain the shortage in the dimpled group. Cases of very faint dimpling run the risk of being classed in the non-dimpled group and thus swell their numbers. That this does occur was proved later on when stray non-dimpled grains gave rise to plants with all grains dimpled, proving that dimpling might fluctuate towards very near non-dimpling and thus escape notice. This practical disability might not altogether explain the aberration in numbers. It is quite possible as in maize [Correns, 1902] that the pollen of dimpled varieties suffers from a handicap in competitive fertilization.

That the ratio obtained in the above table (Table I) is an undoubted 3 : 1, will be obvious from the behaviour of the " 3 " group [Rangaswami Ayyangar, 1934]. In this group, out of the 103 plants obtained from non-dimpled seeds, thirty-four gave heads with all grains non-dimpled and sixty-nine gave hybrids having both non-dimpled and dimpled grains.

The starch grains in non-dimpled and dimpled varieties were examined. The grains are simple and are polygonal in shape. In the non-dimpled varieties the starch grains were larger than in the dimpled varieties. Photographs of starch grains in the dimpled and non-dimpled varieties show this difference clearly (Plate LIII, figs. 3 and 4). Three hundred starch grains in each of the non-dimpled (*A. S.* 158) and dimpled (*A. S.* 219) varieties were measured and the measurements plotted (Fig. 1). It will be seen that the mode for non-dimpled starch grains is at about 26μ, whereas the mode for the dimpled starch grains is about 15μ. Analysis of the dimpled grains, kindly done by the Agricultural Chemist, shows that the amount of reducing sugars in the dimpled grains is thrice the quantity of that contained in the non-dimpled grains, the non-reducing sugars being equal in both.

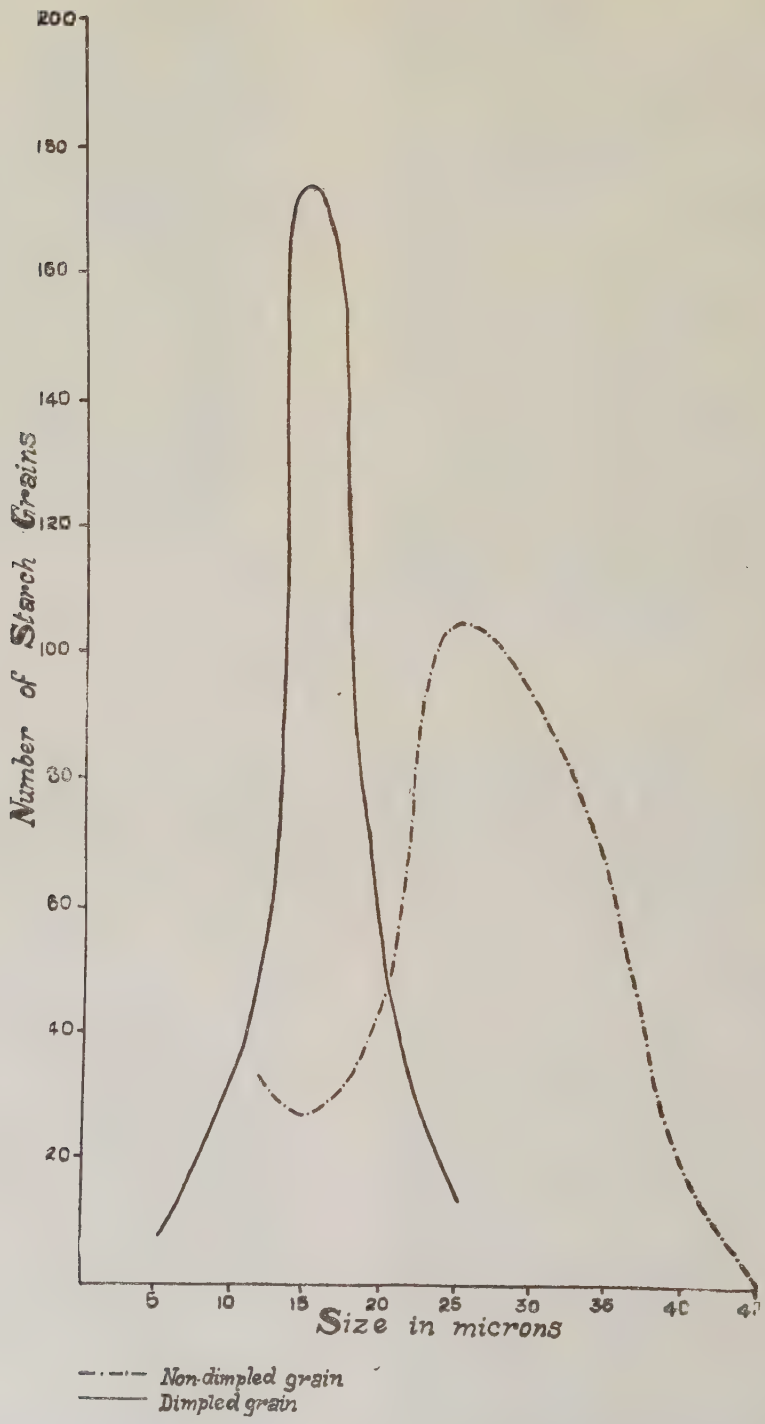

Fig. 1. Size of starch grains from dimpled and non-dimpled seeds.

The dimpled varieties are few in number. The majority of sorghums have non-dimpled seeds. Starch grains in typical non-dimpled varieties and species of sorghum were examined and it was noticed that in all these the starch grains were large and were about 25μ in diameter.

This examination leads one to the conclusion that the starch formation in the dimpled grains is arrested at some stage in their growth, leading to the non-development of the grains to fullness. As the grain ripens and loses moisture, the imperfect development of the starch grains leads to an unfilled cavity inside and the dimple outside.

The Mendelian theory of inheritance postulates random assortment and fertilisation among the gametes. Parnell [1921] first demonstrated that, in rice plants heterozygous for the glutinous character, two types of pollen grains, one staining red when treated with iodine and the other blue, were produced in approximately equal numbers. Demerec [1924], Brink and MacGillivray [1924] in maize, Longley [1924] in *Coix*, and Karper [1933] in sorghum, noted a similar differentiation between the starchy and waxy pollen. The type of dimorphism in pollen, reported herein, belongs to a different category. When treated with iodine the staining was of two shades of blue—light and deep.

An examination of the pollen of dimpled and non-dimpled pure lines was made. Pollen from the dimpled variety (*A.S.* 219) when treated with one per cent iodine stained light blue. In these varieties, the contents of the pollen grains could be made out due to the light stain. The pollen from the normal non-dimpled variety (*A. S.* 158) stained very deep blue in iodine. The whole grain was so intensely blue-coloured that the contents inside the grain could not be made out. Plants heterozygous for dimpling were found to have both kinds of pollen grains, those in which the starch reserve was stained light and those in which it stained deep blue, in about equal proportions on the same head (Plate LIII, fig. 2). The photograph clearly shows the difference in reaction to the staining reagent by the two types of pollen. Counts of pollen grains from ten heterozygous plants gave the expected 1 : 1 ratio.

TABLE II

Pollen counts in hybrid ear-heads

(*Cross No. A. S. CLXXVII*)

Plant No	Total No. of pollen grains	Deep stained pollen	Light stained pollen
1	2,233	1,103	1,130
2	2,394	1,181	1,213
3	2,190	1,096	1,094
4	2,317	1,172	1,145
5	1,181	577	604
6	1,788	919	869
7	1,458	727	731
8	1,673	826	847
9	1,666	858	808
10	1,573	813	760
Total	18,473	9,272	9,201

This dimorphism results in the production of both dimpled and non-dimpled grains in the same ear-head—a clear and easily spotted instance of xenia in sorghum.

The female gametophyte, the embryo-sac, was also examined. The technique adopted was that of Brink [1925] for maize. In the embryo-sac of the dimpled type, a light bluish stain was observed. Non-dimpled embryo-sacs gave the deep blue reaction. Out of the forty-six sacs examined twenty-four gave a deep blue reaction, twenty light blue, and two were doubtful. Under the difficulties of the technique involved in this examination, the almost equal numbers in the two groups are undoubted in their import. The gene resulting in dimpling, it is obvious, exerts the same influence in the female gametophyte as in the pollen. It will thus be seen that there is both pollen and embryo-sac dimorphism manifested in this cross between non-dimpled and dimpled varieties. A factor **dp** is responsible for an arrest in the development of starch grains with the resultant dimple in the mature grain. Factor **Dp** produces the normal well-filled grains of sorghum with the usual size in starch grains. Dimpled grains (**dp**) are a simple recessive to non-dimpled grains (**Dp**).

SUMMARY

Grains with a dimple on them occur in sorghum. Varieties with such grains are rare. Dimpling has been noted in white, yellow, red and brown grains, and in grains that are pearly or chalky in appearance. Dimpling could occur in grains with floury or corneous endosperm. Grains from dimpled varieties are consumed as a delicacy in the dough stage when they are sweet. Dimpled grains contain three

times the amount of reducing sugars found in non-dimpled grains. Dimpling results from the smallness of the starch grains. This smallness is due to the arrest in the development of the starch grains. The pollen grains in dimpled varieties stain light blue and in the common varieties deep blue with iodine. In hybrids between dimpled and common non-dimpled varieties, gametic dimorphism, in both pollen grains and embryo-sacs has been noted. Non-dimpled grains in ear-heads with dimpled grains betray xenia. Dimpled grain occurs frequently in the group *Sorghum cernuum* Host and the dimpled variety has been named *Sorghum cernuum* Host var. *areolatus* Rangaswami. Dimpled grains (**dp**) are recessive to normal non-dimpled (**Dp**) grains.

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THE STORAGE OF FUNGAL CULTURES

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(With one text-figure)

Introduction

The prolonged storage of cultures of fungi presents many difficulties, particularly in hot climates. A temperature not exceeding 25°C. is generally admitted to be desirable, the optimum storage temperature varying from 0° to 20°C. according to the species under consideration. The use of a cellar as culture store may be sufficient to secure this, but if refrigeration is needed, it is desirable to avoid a system which involves either air circulation or an increased humidity of the atmosphere, since both these factors lead to infected cultures.

A damp storage atmosphere increases the risk of infection both by mould fungi and by mites. An atmosphere that is too dry, on the other hand, desiccates the culture media, and necessitates frequent sub-culturing. To try and check evaporation by the use of rubber caps for the tubes is to invite infection, since such caps merely cause the cotton plugs to take up moisture from the medium without excluding atmospheric infection. A better plan, adopted in some culture collections, is to dip the plugged ends of the tubes in melted paraffin wax. The only objection to this method is that the cultures are cut off from the external air supply, whilst vegetative growth still continues, and are thus forced to live amid the accumulation of their own toxic products. Such cultures consequently are liable to abnormal development or early death.

If ordinary cotton plugs are employed, the most serious danger is that of infestation by mites. Many workers are apt to overlook this peril until they have once had experience of it. Mite infestation is not readily noticeable in its early stages. Later, cultures acquire a somewhat moth-eaten or glazed appearance, and mites can be seen on careful examination of the tube under a low power of the microscope. Mites travel from tube to tube, undeterred by the cotton plugs, and leaving bacterial and fungal spores behind them as contaminants. Infection by small-spored fungi, *e.g.*, of the *Cephalosporium* type, is a common indication that mites are at work. A preference is often shown for certain types of culture; *Penicillium* spp. seem to be particularly liable to attack.

As precautionary measures, volatile repellents (*e.g.*, naphthalene) suffer from the objection that they interfere with normal fungal growth. In the writer's experience both pyridine and carbon tetrachloride have been found fairly harmless if their vapour is not too concentrated ; also the occasional application to the plugs of a few drops of one per cent naphthalene in alcohol, or of petrol ether. Some workers [Thom, 1930] dip the plugged ends into a solution containing mercuric chloride. The best course, however, is to avoid mite infection altogether by keeping shelves and incubators clean, by excluding infected material, and by never allowing tubes to lie with their plugs touching the working bench.

Methods of storage other than on agar slope cultures have been tried by various investigators. Fragments of mycelium—with or without agar medium—are said to remain viable if kept in sugar solution. Spores have been preserved for long periods mixed with earth, chopped straw, chalk, or sand. Thaysen [1924] described a method of storage of bacteria on sand in sealed tubes, and in a later paper [Thaysen, 1934] records further data on the viability of both bacterial and fungal cultures preserved by this method. The only fungi recorded are *Penicillium "glaucum"*, *Aspergillus glaucus* and *Rhizopus japonicus*. All were healthy after twelve months storage on sand. After twelve years' storage, spores of the first two species were non-viable, but those of *Rhizopus japonicus* gave rise to an active culture.

Experimental

Sand cultures

Method of preparation.—Purified sand suitable for this purpose may be obtained from most chemical firms, or fine "silver sand" may be purified in the laboratory by warming with hydrochloric acid, washing until free from acid, and then heating in a muffle furnace. Test tubes—preferably rather narrower than the normal variety—are then filled with the treated sand to a height of about one inch from the bottom, plugged with cotton wool, and sterilised for two hours at 160°C.

An appreciable quantity of spores from a healthy culture of the fungus to be preserved is removed by means of a wire loop, and mixed with the sand, the usual precautions being taken to avoid infection during this transfer. Spores are in some cases more easily picked up if the loop is first moistened with sterile water.

The inoculated tubes are incubated at 25°—30°C. in a dry atmosphere for some days in order that the spores may dry off, and are then sealed in the blow-pipe. The worker unaccustomed to glass blowing requires a little practice before he can carry out the sealing process neatly and without failures. The following hints may be useful :—

(1) Employ good quality glassware, not too thin.

(2) Use a foot-blower and a fairly small flame.

(3) Soften thoroughly a small length of the tube and then pull out sharply without, however, sealing the tube (stage 2 in Fig. 1).

(4) Re-soften and seal at the tapering portion, at the same time warming the upper part of the enclosed air space so that the sealed end remains nicely rounded, without either excessive expansion or excessive shrinkage (stage 3 in Fig. 1).

(5) Transfer to the cool flame at the critical moment, then set the tube aside to cool slowly, taking care that the sand is not allowed to come into contact with the hot part of the tube.

The ideal to aim at is to produce a seal indistinguishable from the original end of the tube. Such sealed cultures are very robust, and convenient to handle or to send through the post.

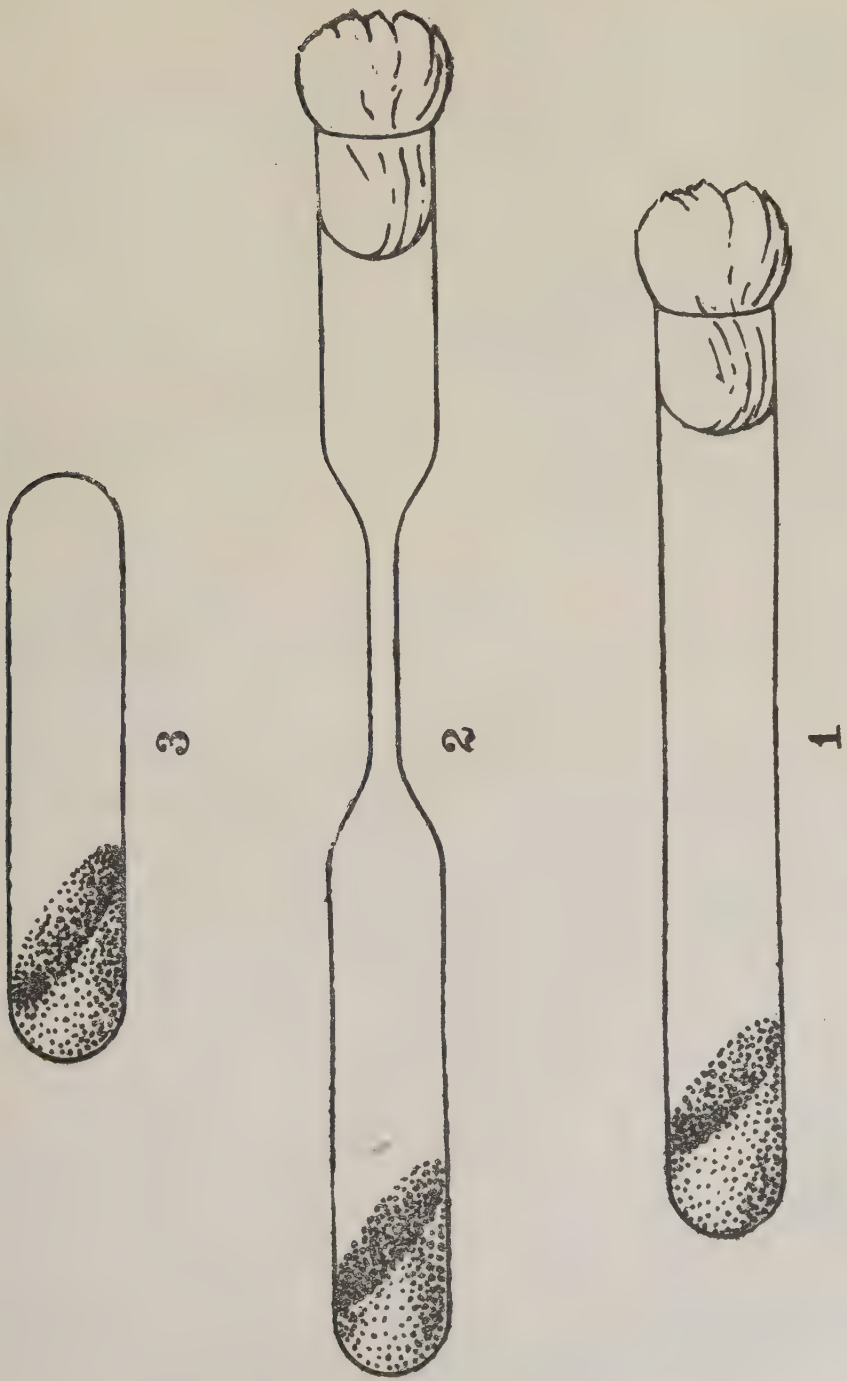

Fig. 1.

Method of sub-culturing.—When it is desired to open a sealed tube, a small file mark is made near the seal. The end of a thin glass rod is then heated to redness in a Bunsen or blow-pipe flame, and pressed firmly against the file mark, with the rod normal to the surface of the glass. The tube should crack all round, and after passing through the flame to sterilise the outer surface, the top may be removed by a gentle tap. The sand is then tipped into a sterile Petri dish, and 20 c.c. of a suitable agar medium, melted and cooled to 42°-45°C. is poured over it. An alternative method that is to be preferred is to tip the sand into a tube of suitable liquid nutrient medium ; about one c.c. of the liquid is then used for plating as before, and the remainder of the liquid, containing the sand, is incubated as a reserve culture in case of failure of the plate culture.

Experimental.—In 1931, sand cultures were made in England of a number of representative mould fungi isolated principally from textiles [Galloway, 1930]. These cultures were opened after two years' storage at normal English laboratory temperatures, and the results are shown in Table I. Authorities for species are omitted for the sake of brevity.

TABLE I

Sand cultures

Species	Viability after two years (or 18 months where indicated with asterisk)
Aspergillus chevalieri, strain A	+
" " " B	+
A. amstelodami	+
A. repens	+
A. nidulans, strain A	+
" " " B	+
A. versicolor, strain A	+
" " " B	+*slow**
" " " C	—*
A. niger, strain A	+
" " " B	+
A. candidus, strain A	+*
" " " B	+*
" " " C	+
A. flavipes	+
A. effusus	+
A. tamarii	+
A. ochraceus	+
Aspergillus sp. 125	+
Penicillium candidofulvum, strain A	+
" " " B	+slow*
" " " C	—
P. chrysogenum, strain A	+
" " " B	+

TABLE I—*contd.*

Species	Viability after two years (or 18 months where indicated with asterisk).
P. expansum	—
P. lilacinum	—
P. pinophilum	—
P. luteum	+
P. purpurogenum	+
P. rugulosum	+
Penicillium sp. 209 B	+
Penicillium sp. 138 G	+
Scopulariopsis brevicaulis	+
Cladosporium herbarum, strain A	+*
,, ,, ,, B	+
Trichoderma lignorum	+*
Rhizopus arrhizus	+*
Rhizopus sp. 6	+*
Mucor sp. 19	+*
Mucor sp. 210 B	+*
Trichothecium roseum	—*
Thamnidium sp. 190	+
Stachybotrys ? atra	+
Stemphylium sp. 207 A	+
,, ,, 207 B	+
Chaetomium sp. 206	+
Memnoniella echinata	+

It will be seen that forty-one out of the forty-seven species survived. Of those that did not survive, *Trichothecium* has thin-walled spores, and the remainder comprise four *Penicillium* spp. and an *Aspergillus*. The results are based on one sealed culture of each type.

The second series of experiments comprises a few sand cultures sealed in England in 1933, and two years later brought to India and opened on arrival. Results are shown in Table II.

TABLE II

Sand cultures

Species	Viability after two years
Aspergillus versicolor	+
A. flavus (in duplicate)	+
A. oryzae	—
A. ochraceus	+
A. candidus	+
Scopulariopsis sp. 221	+
Stachybotrys ? atra	—
Chaetomella sp. 238	+

The third series consists of sand cultures sealed in India in 1935 and opened a year later. A number of plant pathogens were included, and the cultures were stored at Indian laboratory temperatures, which reached 35°C. during the hottest months. Results are shown in Table III.

TABLE III

Sand cultures

Species	Viability after one year
Aspergillus nidulans	+
Trichoderma lignorum, strain A	+
" " " B	—
Colletotrichum falcatum	—
Sclerotium rolfsii (sclerotia)	+
Sclerotium oryzae (sclerotia)	—
Rhizoctonia microsclerotia (sclerotia)	+
Ustilugo hordei	+
U. kolleri	+
U. tritici	—
U. scitaminea	—

Sealed cultures in sugar solution

This is claimed to give good results in some hands, but the exact methods used do not appear to be recorded. The writer's attempts to make use of sugar solutions have been less successful, but two series of experiments are recorded in Tables IV and V.

In the first series, various mould fungi were grown in a nutrient solution (Czapek's) modified to contain ten per cent cane sugar and one per cent chalk. The fungi were allowed to grow until freely sporing and the tubes were then sealed off. These cultures were made in England, taken to India after six months, and opened in India in February 1935 about nine months after the time of sealing. It will be seen from Table IV that two-thirds of the cultures proved non-viable.

TABLE IV

Cane sugar cultures

Species	Viability after nine months
Aspergillus amstelodami	—
A. ruber	—
A. herbariorum minor	+
A. nidulans (non-perithecial)	—
A. niger	+

TABLE IV—*contd.*

Species	Viability after nine months
A. versicolor	+
A. Sydowi	—
A. terreus	+
A. flavipes	+
A. Wentii	—
A. ochraceus	—
A. oryzae	+
A. ustus	—
A. tamarii	—
Penicillium candidofulvum	—
P. pinophilum	—
P. purpurogenum	+
P. rugulosum	—
P. spinulosum	—
Penicillium sp. 239	—
Scopulariopsis brevicaulis	+
Paecilomyces varioti	—
Byssochlamys fulva	+
Mortierella sp. 248	—
Syncephalastrum sp. 192	+
Chaetomella sp. 238	+
Stemphylium sp. 214	—
Stachybotrys ? atra	—
Acrothecium lunatum	—
Stysanus sp. 255	—
Memnoniella echinata	—

A few similar cultures were sealed in India in April 1935 but with further modifications. In the first place, ten per cent dextrose was used in place of ten per cent saccharose, and secondly the transfer made was of a portion of an agar culture containing active mycelium, the tubes being sealed immediately after inoculation. The viability was tested after one year's storage at Indian laboratory temperatures.

TABLE V

Dextrose cultures

Species	Viability after twelve months
Aspergillus nidulans	+
A. niger	—
Trichoderma lignorum, strains A and B	—
Chaetomium indicum	—
Colletotrichum falcatum	—
Phytophthora spp., 4 strains	—

It will be seen from Tables IV and V that both methods of storage in sugar solution gave results inferior to those obtained with sand.

Bacterial cultures

The storage of bacterial cultures does not come in the scope of the present paper, but it may be mentioned for purposes of comparison that the sand culture method is applicable to spore-forming bacteria, the bacterial spores showing even greater viability in this respect than fungal spores.

Non-sporing bacteria, such as the plant pathogenic forms, may be sealed as liquid cultures in broth containing one per cent dextrose with chalk added to maintain a neutral reaction. A culture of *Streptococcus lactis* made in this way was active after $2\frac{1}{2}$ years' storage in England ; of two plant pathogens whose viability was tested after seven months storage in India, a bacterial leaf-streak organism from maize was non-viable, whilst the Red-stripe organism from sugarcane was still active.

Discussion

The results given in this paper indicate that sand cultures afford a useful means of storing certain fungi, in particular those whose spores are thick-walled. It may be anticipated that such spores will retain their viability for a year or two in this state, but sand storage should only be regarded—except for well-tried species—as an additional and not as an alternative means of storing valuable cultures. In interpreting the results shown in Tables I—III, it must be remembered that each record is based on a single culture, and that non-viability may in some cases be due to faulty technique in making this culture, *e.g.*, use of a hot needle, failure to transfer sufficient spores, use of immature cultures, or accidental heating of the sand during the sealing process.

When sand cultures can be relied upon, they present a number of advantages. All chance of infection by mites or moulds is definitely ruled out. The cultures are compact, not easily broken, and seldom require sub-culturing.

A further point—on which few data are yet available—is that sand cultures are more likely to retain their characteristic features than slope cultures. Species such as *Helminthosporium* when kept on agar media for a number of sub-cultures, are apt to show considerable variation or even to produce marked saltants. The presence of toxic substances [Galloway, 1933] and unfavourable physical conditions have been shown to increase the tendency to saltation, and it is reasonable to assume that such conditions are more likely to occur in old vegetative cultures on agar than in dry spores on sand. Additional evidence that saltants are due to staling products is supplied by the fact that they occur more readily in rich media than in media poor in nutrients [Mitra, 1931].

Summary

Results are recorded for the storage of a number of fungi, mainly saprophytic moulds, in sealed culture for one or two years.

Many fungi possessing resistant spores or sclerotia are capable of retaining their vitality for long periods when stored on dry sand in sealed tubes. This affords a method of keeping valuable cultures safe from all chance of infection. Sand cultures alone, however, should not be relied upon.

Two methods of storage in sugar solutions were tested, but gave unsatisfactory results compared with storage on sand.

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BASE-EXCHANGE STUDIES, I

A PRELIMINARY STUDY OF THE EFFECT OF CERTAIN CATIONS SATURATING* THE SOIL'S EXCHANGE COMPLEX, ON ITS PHYSICOCHEMICAL PROPERTIES, AND THEIR RELATION TO THE PLANT GROWTH

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Introduction

The authors [Singh and Nijawan, 1932] while working on *bari* soil observed that there is a marked improvement in the physical condition of the soil on treatment with soluble calcium salts. *Bari* soil in its original state is extremely hard, difficult to plough, and entirely lacking in permeability. It contains eighty-five to ninety per cent of monovalent exchangeable bases, and does not support vegetation. But when this soil is treated with soluble calcium salts, such as calcium chloride or gypsum, its physical properties are modified, and it is able to support a crop of wheat. The question, therefore, arises whether an entire absence of plant growth on these soils is merely due to a direct toxic effect of a high replaceable sodium content, as suggested by Kelley and Brown [1924, 1925] or due to unfavourable physical conditions prevailing in the soil. The replacement of sodium by calcium in the exchange complex brings about a change in the physical condition of the soil, but whether this replacement is also accompanied by such chemical and biological changes as liberate certain plant food materials essential for the growth of the plant, has yet to be determined. It is the object of this paper to report the results of a preliminary investigation on the relationship of different exchangeable cations, such as calcium, magnesium, manganese, potassium, ammonium and sodium saturating the soil's exchange complex with such physical properties as : (1) rate of percolation, (2) water-holding capacity, (3) dispersion coefficient, and (4) hydrogen-ion concentration, on the one hand, and their relationship to plant growth and the plant food material removed by the crop on the other.

*The term ' saturation ' employed in this paper has a restricted meaning. This merely implies that a soil is saturated with regard to a particular base, when it can take no more of that base in the exchange complex with further leachings under the conditions of the experiments.

Experimental

The soil selected for these experiments was *bari* soil from Montgomery district. It was a medium loam, consisting mainly of sodium clay. The analysis of the soil is given in Table I. It is deficient in nitrogen and organic matter, but possesses all other plant food materials in sufficient quantity. Owing to its being deficient in organic matter, the biochemical changes in this soil are of little significance. Besides, such a soil is highly suitable for the purpose of the study outlined above, as the plant food material which is rendered available as the result of cationic exchange is derived mainly from the inorganic complex.

Table I

(*Per cent on air-dried soils*)

	Per cent
1. Mechanical analysis	
Clay	20·16
Fine silt	15·09
Silt	22·14
Fine sand	41·11
Coarse sand	0·72
Gravel	0·35
2. Chemical analysis	
Insoluble residue	76·85
Soluble silica	0·11
Iron (Fe_2O_3)	4·93
Aluminium (Al_2O_3)	8·54
Calcium (CaO)	3·08
Magnesium (MgO)	1·45
Phosphorus (P_2O_5)	0·25
Potassium (K_2O)	1·16
Sodium (Na_2O)	0·87
Organic matter	0·28
Nitrogen	0·03
3. Available analysis	
Phosphates	0·10
Potassium	0·09
4. Exchangeable bases	
Calcium	0·0307
Magnesium	0·0157
Sodium	0·2160
Potassium	0·0412

Preparation of soils saturated with different cations

The soil after being well powdered was passed through a two-mm. sieve. In order to prepare soils saturated with different cations, one hundred grams of the

soil with 150-200 c.c. of warm normal solutions of the chlorides of calcium, magnesium, manganese, potassium, and ammonium was thoroughly shaken, and kept overnight. Next day the soil was transferred to a Büchenr funnel fitted with a filter paper, and filtered under slight but uniform suction and was further leached with 300-400 c.c. of the corresponding salt solutions. The excess of the salt solution was washed down with distilled water. It was observed that soils saturated with potassium, sodium, and ammonium ions could not be leached effectively with distilled water, as owing to their high dispersion clay particles were carried into the filtrate. Consequently, in these cases leaching was done with fifty per cent alcohol. The soils thus leached were dried, crushed and sieved, and were used for the determination of the following physical properties :—

1. Dispersion coefficient.—The dispersion coefficient being the measure of the state of aggregation of soil particles saturated with different cations, was determined according to Keen and Puri's method [1925]. The results tabulated in Table II indicate that soils saturated with divalent bases are less dispersed and form more resistant aggregates than soils saturated with monovalent bases. Of soils saturated with divalent bases, the magnesium soil gives a higher dispersion coefficient than either the manganese or the calcium soil which have about the same dispersion coefficient. Thus soils saturated with manganese and calcium ions are more flocculated than the magnesium-saturated soil.

TABLE II

Description	Dispersion coefficient	Water-holding capacity (moisture as per cent on air-dried soil)	Rate of percolation (time taken by 100 c.c. of water)			Soil reaction (pH value)
				Hrs.	Mts.	
Calcium soil	31·00	43·67	1.	0	25	8·29
			2.	0	27	
			3.	0	27	
Magnesium soil	41·70	39·94	1.	0	41	8·76
			2.	0	63	
			3.	0	81	
Manganese soil	29·40	42·41	1.	0	9	7·36
			2.	0	9	
			3.	0	11	
Ammonium soil	98·00	34·40	1.	1	29	8·62
			2.	2	58	
			3.	3	24	
Potassium soil	99·40	34·50	1.	2	5	9·11
			2.	4	3	
			3.	4	7	
Sodium soil	94·64	29·10	1.	27	32	10·01

2. Water-holding capacity.—The water-holding capacity affords a means of comparing soils on the basis of their capacity to hold moisture, which depends on the pore-space, and colloidal matter present in the soil. Water-holding capacity was determined by Hilgard's method as described by Hall [1921] with modifications of Keen and Raczkowski [1921]. The data in Table II is a mean figure of five determinations made on individual samples, and the variation between the different determinations made on the same soil was not more than 0·5 per cent.

A comparison of the results contained in the above table shows that monovalent ions (K, NH_4 and Na) cause a change in the arrangement of the colloidal material. The soils saturated with divalent bases can hold more moisture than those saturated with monovalent bases. The calcium-saturated soil has the highest water-holding capacity, while the soil saturated with sodium ions has the least. The water-holding capacity goes on decreasing in the order shown below :—

$$Ca > Mn > Mg > K > NH_4 > Na.$$

Water-holding capacity of soil containing different amounts of exchangeable calcium

The results contained in Table III show that the water-holding capacity of a soil increases with an increasing amount of exchangeable calcium in it. The soil having 0·0432 per cent of exchangeable calcium holds 28·91 per cent of moisture, while the same soil containing 0·1824 per cent of exchangeable calcium holds 44·26 per cent of moisture.

Table III

Serial No.	Exchangeable calcium as Ca (percentage on air-dried soil)	Maximum water-holding capacity (moisture as percentage on oven-dried soil)	Rate of percolation (time required for 100 c.c. of water to pass through)	
			Hrs.	Mts.
1	0·0432	28·91	27	55
2	0·0624	31·88	27	32
3	0·0720	32·44	25	36
4	0·0992	33·11	24	12
5	0·1008	34·02	24	3
6	0·1104	34·90	7	41
7	0·1120	34·88	4	5
8	0·1184	35·05	2	39
9	0·1304	35·53	2	19
10	0·1334	36·26	1	54
11	0·1352	38·47	1	5
12	0·1824	44·26	0	26

3. Rate of percolation.—The rate of percolation in these soils was determined by the method described by Bouyoucos [1930], with a slight modification. As the pressure of the tap water is variable, a manometer was introduced to measure the pressure, and in all soils water was allowed to percolate under a definite suction force (33 cm.). The method, though not absolute, is yet capable of giving fairly good comparative results which can be repeated under similar conditions.

Fifty grams of the soil were well shaken with fifty c.c. of distilled water, and then placed on the Büchner funnel to which No. 40 Whatmann's filter paper was fitted. The soil adhering to the sides of the beaker was washed into the funnel with another fifty c.c. of distilled water, and the suction started. In order that the soil system may become settled, and more or less in equilibrium, the suction was continued for about fifteen minutes before measurements were commenced on the rate of flow of water. Meanwhile, the entire amount of water was never allowed to filter through, as a re-arrangement of soil particles causes a change in the rate of percolation.

The results contained in Table II show that the different cations in the exchange complex produce a striking effect on the rate of percolation of water. The order of rate of percolation is $Mn > Ca > Mg > NH_4 > K > Na$.

The time taken for 100 c.c. of water to pass through a layer of fifty grams of soil varied considerably in the case of soil saturated with different cations. For instance it took nine to eleven minutes for 100 c.c. of water to pass through a manganese-saturated soil, twenty-five to twenty-seven minutes through a calcium-saturated soil, forty-one to eighty minutes through a magnesium-saturated soil, 89 to 204 minutes through ammonium-saturated soil and twenty-seven hours and thirty-two minutes to pass through a sodium-saturated soil. Further, it will be observed, that the rate of percolation decreases if repeated percolations are obtained through the same column of the soil without disturbing it. The change is more marked in the case of a magnesium-saturated soil than in the case of manganese or calcium-saturated soils (where there is practically no change), and is still greater in the case of ammonium and potassium saturated soils. This is due to the fact that when water remains in contact with soils saturated with different cations, the magnesium, potassium and ammonium soils become more puddled than soils saturated with manganese and calcium ions, and it is on account of this puddling effect produced in these soils that there is a marked decrease in the rate of subsequent percolations.

Rate of percolation in soils containing different amounts of exchangeable calcium

The rate of percolation was studied in twelve soil samples containing different amounts of exchangeable calcium. From the results given in Table III it will be observed that the rate of percolation increases with increasing amounts of exchangeable calcium, but there is a sudden rise in the rate of percolation in the case

of samples containing 0·1104 per cent of calcium. One hundred c.c. of water take twenty-four hours and three minutes to pass through the soil containing 0·1008 per cent of exhangeable calcium, while the same amount of water takes only seven hours and forty-one minutes to pass through the soil containing 0·1104 per cent of exchangeable calcium. In other words, a slight increase in the amount of exchangeable calcium at that particular stage can bring about a great difference in the rate of percolation. This observation is of great practical importance, as this difference in the rate of percolation is due to complete flocculation of the soil which occurs when it contains 0·1104 per cent of exchangeable calcium. Further, a slight increase over 0·1104 per cent in the amount of exchangeable calcium is responsible for a great increase in the rate of percolation. When the soil becomes saturated with exchangeable calcium, 100 c.c. of water take only twenty-six minutes to percolate through it.

4. pH value.—The pH values were determined by the hydrogen electrode in soil suspensions containing 1 : 2 ratio of soil to water.

The results given in Table II clearly indicate that the nature of the exchangeable cations in the exchange complex greatly affects the pH values of the soil. Thus a soil saturated with sodium ions has a pH value of 10·01, but when sodium is replaced by potassium, magnesium, ammonium, calcium and manganese ions, the pH values are altered to 9·11, 8·76, 8·62, 8·29 and 7·36 respectively.

Crop

Five hundred grams of each of the soils saturated with different cations were placed in glass tumblers, and wheat was sown in them. Altogether there were four repeats of each treatment. It was observed, that the seeds in soils saturated with divalent bases germinated earlier than those in soils saturated with monovalent bases. In the case of sodium-saturated soils only a few seedlings made appearance, which died soon after. Next, the plants in the ammonium-saturated soil began to show signs of exhaustion, the leaves began to turn yellow at the tips and soon after all the plants died. In all the remaining tumblers the plants were healthy and strong, except in the case of those soils saturated with manganese, where the plants were small in size with thin stems and narrow leaves. The following is a statement of the yields of the wheat crop in grams per pot :—

	Soil					
	Ca	Mg	Mn	K	NH_4	Na
Total yield . . .	6·85	6·10	6·00	3·5	*nil*	*nil*
Grain	2·10	1·70	0·78	0·1	*nil*	*nil*
Straw	4·75	4·40	5·22	3·4	*nil*	*nil*

Exchangeable bases

Exchangeable bases were determined in soils saturated with different cations according to Hissink's method [1923] with the modification suggested by Kelley and Chapman [1930]. It was observed that it was not possible to replace the bases completely, especially in the case of manganese, by leaching the soil with 500 c.c. of normal ammonium chloride solution. Therefore, instead of leaching the soil with 500 c.c. of the solution, it was leached with 1,000 c.c. to bring about complete replacement, and the leachings were collected separately in two litre flasks.

From the data of the exchangeable bases given in Table IV it will be observed that they could not all be completely replaced, and ratios of cations saturating the exchange complex to the total bases varied considerably.

TABLE IV

Exchangeable bases : Percentage on air-dried soil

Serial No.	Description of the soil	Before sowing crop						After removal of crop					
		Calcium as Ca	Magnesium as Mg	Sodium as Na	Manganese as Mn	Potassium as K	Ammonium as NH_4	Calcium as Ca	Magnesium as Mg	Sodium as Na	Manganese as Mn	Potassium as K	Ammonium as NH_4
1	Calcium soil . .	0·1824	0·0306	0·0144	...	0·0343	...	0·1504	0·0159	0·0374	...	0·0240	...
2	Magnesium soil .	0·0480	0·1204	0·0087	...	0·0438	...	0·0448	0·0928	0·0275	...	0·0927	...
3	Manganese soil. .	0·0656	*Nil*	0·0173	0·2377	0·0240	...	0·1664	0·0115	0·0550	0·2089	0·0515	...
4	Potassium soil .	0·0592	0·0169	0·0181	...	0·3633	...	0·0384	0·0273	0·0550	...	0·3384	...
5	Ammonium soil .	0·1136	0·0076	0·0371	...	0·0275	0·1170	0·0976	0·0011	0·0245	...	0·0412	0·0648
6	Sodium soil . .	0·0303	0·0186	0·2347	...	0·0326	...	...	...	...	...	...	...

In order to ascertain whether it was possible to bring about a greater replacement of all the bases of the soil by prolonged leaching, the soil in question was leached with 5,000 c.c. of hot normal calcium chloride solution. The amount of exchangeable calcium estimated showed that the amount of calcium absorbed was the same whether the soil was leached with three to four hundred c.c., or five thousand c.c., of the solution. These results are in accordance with the observations made by Gedroiz [1931], Kelley and Brown [1927], and Csiky [1932] who assert that complete replacement cannot take place though prolonged leaching as observed by Mattson [1931] brings considerable change in the exchange complex.

The data of exchangeable bases calculated in gram equivalents are given in Table V. From these it will be observed that sodium in the original *bari* soil is replaced by other bases in almost equivalent amounts. Therefore, it follows, that so far as the replacement of sodium by other bases is concerned, this is governed by the ordinary laws of chemical reaction. The only exception is in the case of an ammonium soil where besides a true chemical reaction probably absorption compounds are also being formed.

TABLE V

Exchangeable bases in gram equivalents (Per cent on air-dried soil), before sowing crop

Serial No.	Description	Calcium as Ca	Magnesium as Mg	Potassium as K	Sodium as Na	Manganese as Mn	Ammonium as NH_4	Total bases
1	Calcium soil .	0·0911	0·0252	0·0088	0·0062	..	..	0·1313
2	Magnesium soil .	0·0240	0·1004	0·0112	0·0038	..	..	0·1394
3	Manganese soil .	0·0328	*Nil*	0·0061	0·0075	0·0870	..	0·1334
4	Potassium soil .	0·0296	0·0139	0·0929	0·0079	..	..	0·1443
5	Ammonium soil .	0·0567	0·0063	0·0070	0·0161	..	0·0650	0·1511
6	Sodium soil .	0·0251	0·0133	0·0083	0·1020	..	..	0·1507

Mineral analysis of crop

When the amounts of various minerals (Table VI) removed by crops from soils saturated with different cations are compared, the following conclusions are arrived at :—

(*a*) Plants remove in excess only that particular base with which the soil is saturated. For instance potassium is removed in excess from a potassium-saturated soil, magnesium from magnesium-saturated soil and so on.

(*b*) The figures of ash percentages show that the amount of mineral matter removed by crops is greatly influenced by the nature of the cations on the exchange complex. The total mineral contents removed by crops from soils saturated with manganese, magnesium, potassium and calcium are 10·04 per cent, 15·74 per cent, 20·04 per cent and 17·05 per cent, respectively.

(*c*) Perkin [1932], while working on the availability of phosphorus in soils saturated with different cations, observed that more soluble phosphates are formed in the case of soils saturated with iron, aluminium, and magnesium, than in the case of soils saturated with hydrogen, calcium, ammonium, and potassium ions, in which the solubility of phosphates is decreased. The amount of phosphoric acid removed by the crop as shown in Table VI, also confirms this observation of Perkin that the phosphate content of crops from a magnesium-saturated soil is greater than the phosphate content of crops from a soil saturated with other cations.

(*d*) The amount of monovalent bases, namely sodium and potassium removed by a crop from soils saturated with different cations increases with an increase in their pH values. The total amount of potassium and sodium removed by crops from a manganese-saturated soil having a pH value of 7·36 is 2·77 per cent, from a calcium-saturated soil with a pH value of 8·29 is 4·72 per cent, from a magnesium-saturated soil with a pH value of 8·76 is 6·25, and in the case of a crop from a potassium-saturated soil with a pH value of 9·11 the amount of these bases is 10·20 per cent.

TABLE VI

Analysis of wheat crop (per cent on air-dried crop)

Serial No.	Description	Ash	Insoluble residue	Iron and aluminium as Fe_2O_3 and Al_2O_3	Calcium as CaO	Magnesium as MgO	Manganese as Mn_3O_4	Sodium as Na_2O	Potassium as K_2O	Phosphorus as P_2O_5
1	Calcium-saturated soil.	17·05	5·820	1·2176	1·4240	0·6971	0·0100	0·8547	3·870	0·5368
2	Magnesium-saturated soil.	15·74	4·160	0·7351	0·7204	1·1920	0·0100	1·3880	4·869	0·6248
3	Manganese-saturated soil.	10·04	2·634	0·5790	0·7456	0·5217	0·0200	0·2876	2·493	0·5637
4	Potassium-saturated soil.	20·40	4·471	0·6388	0·5905	0·5297	0·0120	0·7958	9·414	0·5412

In Table VII all the minerals are expressed as percentages on ash, and for the sake of comparison the average figures of analyses of the mineral contents of a wheat crop from a normal soil depicted as 'percentage on ash' are also given. From these results it will be observed that calcium is not being removed by the crop from the calcium-saturated soil to the same extent as magnesium, manganese and potassium from the corresponding saturated soils. Large amounts of manganese, magnesium and potassium in the soil's exchange-complex induce an excessive absorption of these bases by the crops.

TABLE VII

Mineral contents on 100 grams of ash

Serial No.	Description	Insoluble residue	Iron and aluminium as Fe_2O_3 and Al_2O_3	Manganese as Mn_3O_4	Calcium as CaO	Magnesium as MgO	Phosphorus as P_2O_5	Sodium as Na_2O	Potassium as K_2O
1	Calcium soil .	34·14	7·14	5·85	8·35	4·09	3·25	5·01	22·70
2	Magnesium soil.	26·44	4·67	4·20	4·58	7·57	3·97	8·82	30·94
3	Manganese soil.	26·23	5·77	12·10	7·43	5·20	5·65	2·86	24·83
4	Potassium soil .	22·30	3·18	3·82	2·94	2·64	2·70	3·97	46·97
5	Normal soil .	50·63	5·24	0·58	7·41	4·13	6·86	1·96	24·03

Discussion

It is evident from the results obtained above that the nature and quantity of cations on the exchange complex are responsible for a change in the structure of the soil. The cations are not only responsible for a change in the physical properties of the soil, but they also govern the physico-chemical and chemical processes occurring in it. Therefore, if the introduction of certain cations in the soil complex improves the physical properties of the soil, it does not necessarily follow that it also increases the crop-producing capacity of the soil. For example, the introduction of an ammonium ion in the exchange complex improves the permeability and water-holding capacity of the soil, but the soil thus treated is unable to support the growth of a crop. On the contrary, a potassium soil with a higher pH value and a slower rate of percolation than an ammonium soil shows, is able to support a good crop. Again, a manganese soil is more permeable and has the same water-holding capacity as a calcium soil, but the plants which germinated in it were poor, with weak stems and narrow leaves. The data for ash percentages also show that plants growing in a manganese soil remove the smallest quantity of minerals.

Stability of calcium in the exchange complex

(*a*) It has been observed that when plants are grown on soils saturated with various cations, there is excessive absorption of these bases by the crop. However, calcium from a calcium-saturated soil is not removed to the same extent as other cations, like potassium, magnesium and manganese. This observation brings out the fact that various cations are held up in the soil complex in different ways and that calcium cations are in a more stable combination with the exchange complex than are other cations.

(*b*) The analyses of a manganese-saturated soil (Table IV) show that the amount of exchangeable calcium in the soil increases from 0·0656 to 0·1664 after the removal of the crop. This clearly shows that manganese in a manganese-saturated soil is not sufficiently strongly held in the exchange complex and is being constantly replaced by calcium.

(*c*) Similarly in the case of potassium and magnesium saturated soils, there is a considerable increase in the exchangeable sodium, and decrease in the exchangeable calcium in the soil after the removal of the crop. This shows that in these soils sodium is constantly replacing potassium and magnesium in the exchange complex, or in other words, magnesium and potassium are comparatively loosely held in the exchange complex. Consequently any improvement brought about by these cations will involve only a temporary improvement.

(*d*) With the exception of a potassium-saturated soil, a calcium-saturated soil removes the greatest quantity of minerals. In this connection it has already been pointed out that a high ash percentage is due to an excessive uptake of potassium by the crop which forms more than fifty per cent of the total mineral matter. This fact further shows the superior quality of the crop grown on a calcium-saturated soil.

From the foregoing discussion it follows that the various cations not only affect the physical properties of the soil, but also profoundly modify its chemical properties. Further, an improvement in the physical properties does not necessarily follow an improvement in the chemical properties of the soil. Finally it appears that a calcium soil is the most stable and best suited for plant growth.

Summary and conclusions

1. A preliminary study of the effect of various cations saturating the soil's exchange complex on the physical and physico-chemical properties of the soil is presented.

2. From the results obtained it appears that the physical properties of a soil such as the dispersion coefficient, the water-holding capacity, the rate of percolation and the pH values are greatly influenced by the nature and amount of cations in the exchange complex.

3. A study of the rate of percolation and water-holding capacity of a soil containing different amounts of exchangeable calcium is invariably followed by an increase in the rate of percolation and water-holding capacity of the soil.

4. A definite amount of exchangeable calcium is required to bring about complete flocculation of the soil, and when this amount is reached, there is a sudden increase in the rate of percolation. Further, a slight increase in the amount of exchangeable calcium beyond that point brings about a correspondingly greater increase in the rate of percolation.

5. Sodium and ammonium saturated soils do not support a crop, but soils saturated with calcium, manganese, magnesium and potassium can do so.

6. There is a certain relationship between the plant food material removed by a crop and the nature of the cations saturating the soil's exchange complex.

(*a*) When a particular base is in excess in a soil it is found that crops tend also to remove that base in excess. The amount of such bases removed varies with the nature of the cations. Manganese, magnesium and potassium are removed in double the quantity, and calcium in almost the same amount as is removed by the crop from a normal soil.

(*b*) The availability of phosphorus is increased by the presence of magnesium, and decreased by the presence of calcium and potassium in the soil's exchange complex.

(*c*) The total amount of minerals present in the crop varies with the nature of the cations, and follows the order given below :—

$$K > Ca > Mg > Mn.$$

7. The pH value of the soil is greatly affected by the nature of the cations.

8. The amount of monovalent bases removed by the plant increases with an increase in the pH value.

9. It does not necessarily follow that an improvement in the physical properties of the soil makes it fit for plant growth, as the nature of the cations also in-

fluences the chemical properties of the soil. Soils saturated with calcium ions are more stable and bring about such physical and chemical changes in the soil as are favourable to the growth of a good crop.

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SOME STUDIES ON THE SCLEROTIAL DISEASES OF RICE (*SCLEROTIUM ORYZAE* CATT.) IN THE PUNJAB

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(With Plate LIV)

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1. Introductory

A disease of rice was reported in 1932 from the Rice Farm, Kala Shah Kaku, causing a considerable damage to the rice crop in certain fields. The disease, in addition to causing a marked decline in yield, interfered with the breeding work of the station as many plants died altogether as a result of it in the experimental plots. On examination of the affected plants on the spot the disease was identified as caused by *Sclerotium orzyae* Catt. In the same season specimens of diseased rice plants showing rot of the stem and infected with *Sclerotium orzyae* were also received from Gujranwala, Gurdaspur, Sialkot, Sheikhupura and Kangra (Palampur) districts.

The loss in yield caused by the disease in the affected fields at the Rice Farm, Kala Shah Kaku, varied from five to eighty per cent. In other places the average loss caused by the disease was reported by the local Agricultural

Assistants to vary from five to twenty-five per cent while many fields were free from the disease. From these figures it is not possible to estimate the total amount of loss caused by the disease in the Province but it is apparent that this malady ranks as one of the major diseases of farm crops in the Punjab. The study of the disease was therefore taken in hand. The present paper deals with the symptoms, distribution and perpetuation of the disease in the Punjab and pathogenicity of the causal fungus studied in pot culture and field experiments. In a later publication, it is intended to give factors affecting the incidence and development of the disease and methods of controlling it.

2. Symptoms of the diseases

The most important and general symptom of the disease as observed in the Punjab is Stem-Rot. But considerable variation has been observed with regard to the extent of Stem-Rot and the time of its occurrence.

I. Stem-rot

When the plants are about two to three months old, *i.e.*, about the beginning of September, the leaf-sheaths and leaves at the first, second or third internode from the base of the plant and up to the part where irrigation water reaches, begin to wither and ultimately dry up. The sheaths soon turn somewhat dark and begin to rot. At this time sclerotia of the fungus appear as small black dots at the bases of dried leaves and within the leaf-sheaths. Then the stem below the diseased sheaths gets infected. Generally the node from where the diseased leaves arise is first attacked. At first a small darkish spot appears and then infection spreads upward and in some cases downward also, and involves the whole internode below the affected leaf-sheath. The infected part of the stem then begins to rot and becomes so soft that it collapses and the plant falls down. In severe cases, it occurs either before the emergence of ears or when the ears have just come out. In such cases, the affected shoot dies altogether or if it remains green, the ears remain totally or partially sterile. If the Stem-Rot appears a little later, grains may be formed, but they are very much shrivelled. If the attack is very late, normal grains may be produced. Thus the extent of the damage depends upon the time when the Stem-Rot sets in (Plate LIV). In all these cases sclerotia of the fungus appear in large numbers inside the affected stem.

II. Drying of basal leaves

In some cases leaf-blades and leaf-sheaths dry on account of the disease but the stem does not rot. In such cases almost normal grains are produced and no appreciable damage to the grains is caused. The sclerotia of the fungus are, however, produced on dried leaf-sheaths.

(*a*) Showing rot of the first internode of a rice plant.
(*b*) ,, ,, ,, second internode of a rice plant.
(*c*) ,, ,, ,, third internode of a rice plant.
(*d*) Showing Sclerotia of '*Sclerotium oryzae*' on the base of a leaf sheath.
(*e*) Showing Sclerotia of '*Sclerotium oryzae*' inside the affected stem of a rice plant.

III. Discolouration of bases of stems

A large number of cases have also been observed where neither the leaves are attacked nor Stem-Rot appears, but the bases of stems of plants, all the same, show discolouration and bear sclerotia. In such cases the plant generally does not collapse. The affected plant either wilts or if it remains green it produces ears which remain sterile or contain shrivelled grains. If, however, the attack is late normal grains are produced.

IV. Presence of sclerotia on stubble

Many cases have also been observed when no symptoms of the disease were noticeable in the standing crop, but after harvest sclerotia of the fungus were found in abundance on stubble.

Excessive and late tillering as described by Butler [1913] and Shaw [1913], whose observations were confined to Bengal, Bihar and Burma, have not been noticed so far in the Punjab, though as above stated, sterility and discolouration of the bases of plants as described by them is fairly common in the Punjab. The Stem-Rot which is the most important symptom of the disease in the Punjab has not been recorded by them. Mundkur [1935], the latest worker on this disease at Pusa (Bihar) whose study is limited chiefly to Pusa and its surroundings and who also visited Anakapalle, Samalkota, Maruteru, Coimbatore and Pattambi in the Madras Presidency and Karnal in the Punjab, did not observe any of the symptoms of the disease though he found sclerotia of the fungus in many plants. It is, however, interesting to note that as in the Punjab, stem-rot is the most important symptom of the disease in many countries abroad as described by Cattaneo [1879] and Briosi and Cavara [1886] in Italy, Reyes [1929] in Philippines, Vincens [1923] in Indo-China, Tisdale [1921] in Louisiana (U. S. A.), and Tullis and Cralley [1933] in Arkansas, Texas and Louisiana (U. S. A.). The nature of damage caused by the disease in these countries is also identical with the Punjab. Park and Bertus [1932] state that in Ceylon the disease causes death of seedlings, and the advanced plants when affected produce abnormal tillers, and the stalk gradually turns yellow and dies.

3. Distribution of the disease

During the last three years, specimens of diseased rice plants affected with *Sclerotium oryzae* and showing the Stem-Rot symptom have been received from Sheikhupura, Gujranwala, Gurdaspur, Sialkot, Palampur and D. G. Khan districts. In 1935, after Mundkur [1935] published his paper on this disease and stated that he did not notice Stem-Rot symptom of the disease in Bihar, important rice-growing tracts in the plains of the Punjab were visited to make further observations on the disease. The disease exhibiting acute Stem-Rot was found fairly common in Sheikhupura, Gujranwala and Karnal districts, but it was not observed in Hoshiarpur and Muzaffargarh districts. The other places were not visited.

It is interesting to remark here that Mundkur visited Karnal in the Punjab but did not find the Stem-Rot symptom of the disease though he found sclerotia of the fungus in leaf-sheaths of plants which had well filled up panicles. This seems to be due to the fact that in Karnal district, the disease is serious in certain parts only, *e.g.*, south-eastern villages near Karnal along the bank of the Western Jamna Canal where in 1935 it caused about thirty per cent damage in certain fields, while in other parts of the district the disease is either absent or is found in traces only. Mundkur probably happened to visit those places in the Karnal district where the disease exhibiting Stem-Rot symptom was absent.

4. Infection experiments

I. Material and methods

In the Punjab, rice is grown by sowing the seed directly and also by transplanting seedlings. For infection experiments, rice was grown by both these methods. Nineteen varieties of rice were included in these experiments. The seed of all the varieties was obtained from the Assistant Cerealist, Rice Farm, Kala Shah Kaku, District Sheikhupura, Punjab. Before sowing, the seed was disinfected in 0·1 per cent mercuric chloride solution for fifteen minutes. The infection experiments were conducted both in pots and field.

For infecting the soil, the infected rice stubble and pure culture of the fungus were mixed thoroughly in the upper three-inch layer of soil. The infected rice stubble was obtained from the Rice Farm, Kala Shah Kaku, and before mixing it with the soil, it was chopped up into fine pieces. The culture of the fungus used was grown on oatmeal agar (Oatmeal 50 grms. and agar-agar 20 grms. made up to one litre in distilled water) at 30°C. The culture used was always fifteen to twenty days old. The direct inoculation of rice plants was done by placing a bit of mycelium along with sclerotia of the fungus on the part of the plant to be inoculated and then tying cotton wool over it which was kept moist for fourteen days. Both pot and field experiments were conducted at Lyallpur and canal water was used for irrigation.

II. Pot experiments

(*i*) *By sowing the seed directly in pots*

Forty flower pots filled with clay soil were sterilized in an autoclave at 120°C. for one hour. The soil of ten pots was infected with diseased rice stubble and of ten more with pure culture of the fungus on 20th May 1933. The remaining twenty pots were left as control. Disinfected rice seed of Punjab variety No. 349 was sown, six seeds in each pot, on 1st June 1933. All the pots were watered thrice daily till the crop matured. The plants were kept under constant observation throughout this period. The results are given in Table I.

The experiment could not be arranged in 1934 but was carried out in 1935, when the soil was infected on 1st June and the seed was sown on 5th June. These results are also given in Table I.

TABLE I

Results of infection experiments in pots by sowing the seed

Year	Treatment	Total No. of plants	No. of diseased plants			
			Showing Stem-Rot symptoms	Showing drying of leaves at the bases of plants	Total	Per cent infection
1933	Soil infected with diseased rice stubble.	57	2	3	5	8·8
	Soil infected with pure culture of the fungus.	56	3	5	8	14·3
	Soil uninfected (control) .	112	0	0	0	0
1935	Soil infected with diseased rice stubble.	52	4	4	8	15·4
	Soil infected with pure culture of the fungus.	51	2	3	5	9·8
	Soil uninfected (control) .	108	0	0	0	0

(*ii*) *By transplanting*

(*a*) *By raising the seedlings in uninfected soil.*—This experiment was carried out in the same manner as (*i*) above, with the only difference that, instead of seed, forty days old seedlings raised in sterilized pots were transplanted on 10th July 1933 in forty pots (ten infected with diseased rice stubble, ten infected with culture of the fungus and twenty uninfected).

The experiment was carried out in 1934 and 1935 also. Thirty-five days old seedlings were transplanted on 10th July 1934, and thirty-seven days old seedlings were transplanted on 12th July 1935. The results of experiments of 1933, 1934 and 1935 are given in Table II.

(*b*) *By raising seedlings in infected soil.*—This experiment was conducted as (*ii*) (*a*) above, with the difference that seedlings were raised in pots the soil of which was infected with pure culture of the fungus just before sowing the seed.

The experiment was carried out in 1933 and 1934 and the results are given in Table III.

TABLE II

Results of infection experiments by transplanting (seedlings raised in uninfected soil)

Year	Treatment	Total No. of plants	No. of infected plants			
			Showing Stem-Rot symptoms	Showing only drying of leaves at the bases of plants	Total	Per cent infection
1933	Soil infected with diseased rice stubble.	55	3	6	9	16·4
	Soil infected with pure culture of the fungus.	53	4	2	6	11·3
	Soil uninfected (control) .	115	0	0	0	0
1934	Soil infected with diseased rice stubble.	54	4	4	8	14·8
	Soil infected with pure culture of the fungus.	56	3	4	7	12·5
	Soil uninfected (control) .	114	0	0	0	0
1935	Soil infected with pure culture of the fungus.	58	2	19	21	36·2
	Soil uninfected (control) .	60	0	0	0	0

TABLE III

Results of infection experiments by transplanting (seedlings raised in infected soil)

Year	Treatment	Total No. of plants	No. of diseased plants			
			Showing Stem-Rot symptoms	Showing only drying of leaves of plants	Total	Per cent infection
1933	Soil infected with diseased rice stubble.	52	3	4	7	13·5
	Soil infected with pure culture of the fungus.	56	2	2	4	7·1
	Soil uninfected (control) .	57	0	2	2	3·5
1934	Soil infected with diseased rice stubble.	53	4	5	9	17·0
	Soil infected with pure culture of the fungus.	54	4	4	8	14·8
	Soil uninfected (control) .	53	1	1	2	3·8

(iii) By direct inoculation

The seedlings of rice raised in uninfected soil were transplanted in pots containing healthy soil on 12th July 1935. The stems of these plants were inoculated with pure culture of the fungus at the base, and also at heights varying from half an inch to two inches above the base on 7th September 1935 and 21st September 1935. Observations were recorded on 20th October 1935 and the results are given in Table IV.

TABLE IV

Result of direct inoculation experiments

Date of inoculation	Part inoculated	Total No. of plants	No. of diseased plants			
			Showing Stem-Rot	Showing drying or rotting of leaves	Total	Per cent infection
7th Sept. 1935.	Soil infected . . .	58	2	19	21	36
	Bases of stem. . .	24	4	4	8	33
	Stem 1½ in. above the base.	25	1	4	5	20
	Control	20	0	0	0	0
21st Sept. 1935.	Soil infected . . .	23	1	3	4	17
	Bases of stem . . .	25	5	4	9	36
	Stem one in. above the base	25	2	15	17	68
	Stem two in. above the base.	25	8	7	15	60
	Control	24	0	3	3	12

Results

In all the above pot experiments the results of which are given in Tables I to IV the fungus was isolated from diseased plants and was always found to be *Sclerotium oryzae* and the diseased plants showed characteristic Stem-Rot symptoms or the drying of basal leaves. These experiments therefore prove that the fungus *Sclerotium oryzae* is pathogenic on rice plants and causes Stem-Rot or drying of basal leaves.

III. FIELD EXPERIMENTS

(i) By sowing the seed directly in the field

A piece of land was divided into four plots, each measuring 30 ft. × 20 ft. The soil of the first and third plots was infected on 15th January 1933 with diseased rice stubble and pure culture of the fungus respectively. Disinfected seed of nineteen varieties was sown on 1st June 1933 in every plot. In each plot there were two sets of nineteen lines each and in each set every variety was sown in one line each.

The experiment was repeated in 1934, when again soil of the first and third plots was infected with diseased stubble and pure culture of the fungus on 15th May. The seed was sown on 5th June. The results of experiments of both the years are given in Table V.

(ii) By transplanting

(a) By raising the seedlings in uninfected soil.—This experiment was conducted in the manner as III *(i)* above with the difference that six-weeks old seedlings of nineteen varieties raised in another piece of uninfected land were transplanted in the four plots on 10th July 1933. The experiment was repeated in 1934 when the seedlings of the same age were transplanted on 15th July.

In 1935 the experiment was conducted again. One portion of a healthy plot was infected with pure culture of the fungus on 1st July and the other was left as control. Five-weeks old seedlings of nineteen varieties were transplanted in these plots on 15th July. The results of all the three years' experiments are given in Table VI.

(b) By raising the seedlings in infected soil.—The plan for this experiment was the same as for *(ii)* *(a)* above with the difference that the seedlings for transplanting were grown in soil which was infected with pure culture of the fungus. The experiment was carried out for two years, 1933 and 1934. The results are given in Table VII.

TABLE V

Results of infection experiments in the field by sowing seed

Serial No.	Variety	Percentage infection							
		1933				1934			
		Diseased stubble added	Healthy soil	Culture of fungus added	Healthy soil	Diseased stubble added	Healthy soil	Culture of fungus added	Healthy soil
1	14	27·5	0	11·7	0	9·1	0	54·6	1·8
2	225	0	0	12·6	0	9·1	0	0	0
3	46	0	0	0	0	4·3	0	31·0	0
4	349	5·0	0	1·7	0	4·1	0·5	48·3	0
5	346	0	0	0	0	6·7	0	9·4	0
6	22	0	0	3·0	0	0	0	0	0
7	7	0	0	4·7	0	0	0	0	0
8	278	4·0	0	5·1	0	3·4	0	18·3	0
9	K. V. 22/28	0	0	0	0	7·9	0	0	0
10	K. V. 38A/28	0	0	0	0	26·2	0	0	0
11	360, 370, 3/30, 337A, 41, 202, 62, 12(S) K. V. 21/28.	0	0	0	0	0	0	0	0

TABLE VI

Results of infection experiments in field by transplanting seedlings growing in uninfected soil

Serial No.	Variety	Percentage infection									
		1933				1934				1935	
		Diseased stubble added	Healthy soil	Culture added	Healthy soil	Diseased stubble added	Healthy soil	Culture added	Healthy soil	Culture added	Healthy soil
1	14	5·0	0	0	0	41·7	2·1	80·1	8·0	29·2	0
2	225	17·5	1·0	6·2	0	9·1	0	31·0	0	43·1	0
3	46	0	0	0	0	4·3	0	39·9	0	11·7	0
4	349	11·4	0	5·7	0	3·5	0	48·3	4·5	19·4	0
5	346	0	0	0	0	6·5	0	10·1	0	0	0
6	360	0	0	0	0	0	0	0	0	25·0	0
7	22	7·1	0	0	0	0	0	0	0	35·5	0
8	3/30	0	0	0	0	0	0	0	0	2·1	0
9	337A	7·5	0	0	0	0	0	0	0	5·5	0
10	62	0	0	0	0	0	0	0	0	6·7	0
11	12(S)	0	0	0	0	0	0	0	0	33·1	0
12	278	13·1	0	0	0	0	0	23·5	3·5	26·1	0
13	K. V. 22/28	25·0	0	0	0	7·9	0	37·0	0	15·0	0
14	K. V. 21/28	0	0	0	0	0	0	0	0	9·7	0
15	K. V. 38A/28	33·3	0	0	0	4·0	0	0	0	16·3	0
16	370, 41, 7, 202	0	0	0	0	0	0	0	0	0	0

TABLE VII

Results of infection experiments in field by transplanting seedlings grown in infected soil

Serial No.	Variety	Percentage infection 1933				Percentage infection 1934			
		Diseased stubble added	Healthy soil	Culture added	Healthy soil	Diseased stubble added	Healthy soil	Culture added	Healthy soil
1	14	35·9	16·1	33·8	0	30·0	0	10·3	0
2	225	15·4	18·2	36·8	1·0	36·2	0	55·7	2·0
3	46	17·3	8·1	20·0	0	28·0	0	20·0	0
4	349	21·8	12·5	33·9	0	40·9	0	43·7	0
5	346	0	0	0	0	17·2	0	3·7	0
6	360	2·9	0	0	0	8·0	0	0	0
7	22	2·4	0	6·2	0	11·1	0	25·1	0
8	370	0	0	1·5	0	10·2	0	0	0
9	337 A	0	0	0	0	3·4	0	3·5	0
10	41	1·2	0	0	0	3·5	0	3·5	0
11	12 (S)	1·2	0	0	0	0	0	5·5	0
12	278	16·4	13·8	40·0	0	75·4	14·9	37·5	0
13	K. V. 22/28	7·5	18·1	25·0	0	30·0	0	54·0	0
14	K. V. 21/28	1·5	0	2·6	0	0	0	22·2	0
15	K. V. 38A/28	39·9	1·0	27·2	0	36·2	0	55·7	0
16	3/30, 7, 202, 62	0	0	0	0	0	0	0	0

Results

From the results embodied in Tables V-VII the following conclusions can be drawn.

1. *Sclerotium oryzae* is parasitic on rice plant and causes Stem-Rot disease under field conditions at Lyallpur (Punjab).

2. The disease is produced whether seed is sown directly or whether seedlings at first grown in uninfected soil are transplanted in land infected with diseased rice stubble or pure culture of the fungus.

   The disease is also produced, though to a smaller extent, if seedlings grown in infected soil are transplanted in uninfected soil.

3. The different varieties of rice differ markedly as to their relative susceptibility to the disease.

It may be remarked here that, under Pusa (Bihar) conditions, Mundkur [1935] got negative results of infection experiments in field as well as pots. This is probably due to the difference in climate, nature of soil and other cultural practices between the Punjab and Bihar.

5. Methods of perpetuation of the disease

Observations made in different localities of the province showed that the rice crop grown on land, which had borne a crop affected with *Sclerotium oryzae* the previous year, is attacked by the disease. To test this the following experiments were arranged both at Lyallpur and Kala Shah Kaku.

I. Experiments at Lyallpur

In the first and third plots referred to above under infection experiments (4, III), no further inoculum was added after harvesting the crop in 1934.

In 1935 rice variety No. 349 was grown in these plots by sowing disinfected seed in one half on 1st June and transplanting six-weeks old seedlings raised in uninfected soil in the second half on 15th July. Similar sowings were also done in a plot which had carried a healthy rice crop the previous year to serve as control. The results are given in Table VIII.

Table VIII

Results of an experiment of growing rice in naturally infected land at Lyallpur in 1935

Treatment	Seed sown			Seedlings transplanted		
	Total No. of plants	Infected plants	Per cent infection	Total No. of plants	Infected plants	Per cent infection
Infected land (Diseased crop in 1934)—						
Plot I	144	53	36.8	78	38	48.7
Plot II	109	41	37.6	90	45	50.0
Plot III	96	45	46.8	83	28	33.7
Plot IV	128	8	6.2	87	6	6.9
Plot V	116	11	9.5	86	8	9.3
Total	593	158	26.6	424	125	29.5
Healthy land	531	17	3.2	402	4	1.0

II. Experiments at Kala Shah Kaku

A field in which there were about eighty per cent diseased rice plants showing acute Stem-Rot in 1932 was reserved for the experiment. In 1933 seedlings of nineteen varieties were transplanted in this field. Again in 1934 the same nineteen varieties were grown in this field by transplanting seedlings. The seedlings were raised in an apparently healthy soil, but as the disease is widely present at the farm, freedom of the nursery bed from infection cannot be guaranteed. For the same reason the control plot could not be arranged. The results are given in Table IX.

It is clear from the results given in Tables VIII and IX that if rice is grown in land which carried a diseased crop the previous year the disease is produced.

Table IX

Results of experiments of growing rice in naturally infected land at Kala Shah Kaku, district Sheikhupura, in 1933 and 1934

Serial No.	Variety	1933			1934		
		Total No. of plants	No. of infected plants	Percentage infection	Total No. of plants	No. of infected plants	Percentage infection
1	14	740	589	80·0	829	798	96·6
2	225	561	356	63·5	660	448	68·0
3	46	729	90	12·5	573	281	49·5
4	349	735	545	74·0	823	656	79·8
5	346	812	554	68·0	659	557	84·6
6	360	769	445	60·0	670	587	87·6
7	22	555	111	20·0	662	227	34·3
8	370	805	52	6·4	593	118	20·0
9	3/30	835	74	8·9	659	544	82·5
10	337A	435	237	54·5	599	441	73·6
11	41	761	297	39·0	642	329	51·2
12	7	722	50	7·0	658	134	20·3
13	202	582	367	63·0	654	476	72·7
14	62	628	34	5·4	670	174	26·0
15	12 (S)	707	454	64·0	647	601	92·7
16	278	664	604	91·0	897	786	87·6
17	K. V. 22/28	771	201	26·2	658	348	52·1
18	K. V. 21/28	596	17	3·0	544	348	64·0
19	K. V. 38A/28	289	14	5·0	452	162	35·8

A comparison of Table IX with Tables V-VIII shows that the incidence of the disease is much higher at Kala Shah Kaku than at Lyallpur. This may be due to differences in the nature of the soil, etc., between the two places.

6. Summary

1. Symptoms of the disease of rice caused by the fungus *Sclerotium oryzae* have been described. The most important symptom of the disease in the Punjab is Stem-Rot.

2. The disease is common in many rice-growing districts of the Punjab and causes considerable damage.

3. Inoculation experiments have been carried out in pots as well as in the field. These prove that *Sclerotium oryzae* is parasitic on rice plant both under pot and field conditions in the Punjab.

The disease is produced whether seed is sown directly or whether seedlings at first grown in uninfected soil are transplanted in land infected with diseased rice stubble or pure culture of the fungus.

The disease is also produced, though to a smaller extent, if seedlings grown in infected soil are transplanted in uninfected soil.

4. Rice grown on land which carried a diseased crop the previous year gets diseased.

5. Varieties of rice differ markedly as to their relative susceptibility to the disease.

7. Acknowledgments

The writers feel pleasure to acknowledge the help rendered by Ch. Chetan Swarup, Agricultural Assistant, who looked after the field experiments during the 1935 season. They are also grateful to Khan Sardar Khan, Assistant Cerealist, for supplying seed of different varieties of rice and for the facilities provided for growing rice at the Rice Farm, Kala Shah Kaku.

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PHOTO-CHEMICAL OXIDATION BY PLANT MATERIALS

BY

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Introduction

In course of investigations on photo-chemical oxidations of ammonia and similar nitrogenous compounds, Dhar and his co-workers have shown that many inorganic compounds like titania, zinc oxide, etc., act as photo-catalysts. In experiments reported from this laboratory [Fazal-ud-Din, 1935], organic constituents of the soil were found to exert an oxidizing influence. Ordinarily one would expect the organic matter to exert a reducing influence, therefore the oxidizing influence of the organic residues, however small, is of interest in the proper understanding of these processes in soil. It was further found that fresh plant materials also exerted similar influence. The absorption of nitrogen in the form of ammonia by most of the plants has been mainly considered improbable because the mechanism of the oxidation of ammonia inside the plant tissues is not explainable by present-day conceptions of plant physiology. The experiments carried out in this laboratory throw some light on how this oxidation can be brought about in the plants. Further the formation of proteins by interaction of the oxidized nitrogen and sugars has been demonstrated in presence of oxidizing catalyst, namely, titania by Eggleton [1935] and Dhar *et al.* [1934] *in vitro*. If the formation of proteins by similar interaction be postulated as occurring *in vivo*, the role of titania in the experiment *in vitro* can be easily assigned to particular organic plant catalysts in the similar transformation *in vivo*, as both these act as similar oxidizing photo-catalysts.

In course of these experiments it was found that the organic matter derived from various sources and from varied plants, fresh as well as decayed, exerted an oxidizing influence on ammoniacal compounds. The scope of the present investigation has been limited to a detailed examination of the plant tissues of one crop only. Berseem (*Trifolium alexandrinum*) was chosen for these studies.

Method

Fifty c.c. of one per cent mono-ethylamine solution and the desired amount of the catalyst were put in Pyrex conical flasks of 300 c.c. capacity. The flasks were plugged with cotton wool and exposed to the sun. In case the flasks were to be sterilized, mono-ethylamine was added after sterilization with a sterile pipette. Nitrous nitrogen was estimated by Griess Ilosvay's colorimetric method.

(1) Fully grown plants were up-rooted. These were washed freely first with tap water, and then with distilled water. The air-dried plants were ground and passed through two mm. sieve. The catalytic power of 0·5 grm. of the ground material was determined. The flasks were not sterilized. Dark controls were kept side by side with the exposed flasks. The results are given in Table I.

Table I

Showing the catalytic action of powdered berseem

Serial No.	Treatment	Mg. nitrous nitrogen per 100 c.c.
1	Exposed with the catalyst	0.065
2		0·065
3	Unexposed with the catalyst	*Nil*
4		*Nil*
5	Exposed without the catalyst	0.004
6		0.006

(2) To investigate whether the catalytic principles were heat-stable, half a gram of the material was taken in each of the four flasks containing fifty c.c. of water. Two flasks were heated in an autoclave for half an hour at 125°C. The catalytic power was determined in the usual way. The results (Table II) showed that these catalysts were heat-stable and that the oxidation observed was not due to the enzymatic activities of substances present in the plant material and other bacterial decomposition.

Table II

Showing the action of heat on the catalytic action of berseem

Serial No.	Treatment of the catalyst	Mg. nitrous nitrogen per 100 c.c.
1	Unheated	0.078
2	,,	0.068
3	Heated	0.065
4	,,	0.065

In order to find out whether the catalytic power was confined to a particular tissue or more diffused in nature, the parts like leaves, stems, and roots were separately sampled and their catalytic power was determined. Further, these samples were separately extracted with ether in Soxhlet extraction apparatus for twelve hours. The residues were further extracted with cold and hot water successively. The catalytic power of the different tissues and their extracts corresponding to 0·5 grm. of the substance was determined. The results are given in Table III.

TABLE III

Showing the catalytic action of different tissues and their extracts

Serial No.	Treatment	Mg. nitrous nitrogen per 100 c.c.		
		Roots	Stems	Leaves
1	Untreated	0.069	0.033	0.053
		0.049	0.043	0.072
2	Ether extract	0.016	0.014	0.016
		0.016	0.020	0.016
3	Cold water extract	0.039	0.014	0.039
		0.039	0.017	0.039
4	Hot water extract	0.017	0.007	0.020
		0.017	0.007	0.020

These show that :—

1. The catalytic power of the leaves and roots was more than that of stem.
2. The ether extracts of all the tissues were catalytic to the same extent.
3. The water extracts were found to be much better catalysts.
4. It appeared that the catalytic agents were almost completely extractable, as the total oxidation by the three made up the oxidation by the untreated material.

Crude fibre showed very slight catalytic action ; pure cellulose prepared from cotton wool did not show any.

As most of the catalytic power was confined to the water extracts, it was considered that the role of soluble organic materials may be of importance in this process. Therefore the catalytic power of different carbohydrates was determined. The results (Table IV) showed that fructose, glucose and maltose acted as positive catalysts.

TABLE IV.

Showing the catalytic action of the carbohydrates

Serial No.	Catalyst	Mg. nitrous nitrogen per 100 c.c.	
		N/5 ammonia	One per cent mono-ethylamine
1	Fructose	Trace	0.023
		,,	0 019
2	Sucrose	Trace	0.007
		,,	0.007
3	Glucose	0.004	0.013
		0.005	0.019
4	Maltose	0.005	0.034
		Trace	0.036
5	Starch	,,	0.008
		,,	0.008
6	Control	,,	0.008
		,,	0.008

For further confirmation of the catalytic action of glucose, five grams of starch was taken in each of the two flasks. One hundred c.c. of water was added in each. In one flask two c.c. of extra-pure hydrochloric acid was added. Both the flasks were heated for half an hour in an autoclave at 120°C.

Twenty c.c. of the emulsion was titrated with phenolphthalein as an indicator, and the amount of soda required to neutralize the acid was determined. The catalytic power of one gram of the hydrolysed starch was determined after neutralization of the free acid with sodium hydroxide. In the control flasks the equivalent amount of sodium chloride was added.

TABLE V

Showing the catalytic action of hydrolysed starch

Serial No.	Catalyst	Mg. nitrous nitrogen per 100 c.c. solution
1	Starch + sodium chloride	0.010
		0.010
2	Hydrolysed starch + sodium chloride	0.052
		0.055
3	Without any catalyst	0.005
		0.005

The results given in Table V showed that such strong reducing agent as glucose acted as positive oxidizing catalyst and that this was conclusively shown

to be due to the sugar and not to any microscopic impurity that may have been present in the extra-pure sugar employed.

The role of the green colouring matter of the plants in the oxidation process was further studied. For this purpose 0·1 grm. of pure preparations of crystalline and amorphous chlorophylls were used separately for determining the catalytic action. The results showed that both the substances had considerable catalytic power (Table VI).

TABLE VI

Showing the catalytic action of chlorophyll

Serial No.	Catalyst	Mg. nitrous nitrogen per 100 c.c.
1	Amorphus chlorophyll (phytyl)	0.071 0.064
2	Crystalline chlorophyll (ethyl)	0.064 0.052
3	Without the catalyst	0.005 0.005

The catalytic power of the tissues of berseem was unaffected by the age of plants as tissues derived from plants growing for different lengths of time gave almost the same catalytic value. The seeds of berseem plants however were found to have a slightly stronger catalytic action than that of other tissues.

Further it was considered likely that carbon dioxide may be evolved during the course of photo-chemical reaction, therefore the effect of carbon dioxide on this process was investigated. For this purpose four c.c. and eight c.c. of pure carbon dioxide gas was introduced in 300 c.c. flasks containing fifty c.c. of *N*/5 ammonium sulphate solution and one gram of zinc oxide and sealed off. The results (Table VII) showed that carbon dioxide had considerable depressing effect.

TABLE VII

Showing the effect of carbon dioxide on the oxidation of ammonium sulphate

Serial No.	Treatment	Mgs. of nitrogen oxidised per 100 c.c.	
1 2	Control	6.4 9.3	7.8
3 4	4 c.c. CO_2	5.9 5.1	5.5
5 6	8 c.c. CO_2	3.4 2.2	2.8

It has been observed that nitrogen can be assimilated in the ammoniacal form by plants under certain conditions. Eggleton [1935], while studying the transformation of various forms of inorganic nitrogen applied to the soil, found that the amount of nitrite present in the grass leaves increased two hours after the application of ammonium sulphate to the soil. This showed that the ammonium slats were oxidised in the plant. The results reported in this paper indicate how such oxidation is possible by photo-chemical means.

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ABSTRACT

An account of the occurrence of *Chrysomphalus aurantii* Mask. and *Laccifer lacca* Kerr. on grape fruit in Ranchi District, Chota Nagpur, with a note on the *Chalcidoid* parasites of *Aspidiotus orientalis* Newst. P. M. GLOVER (*Journal of the Bombay Natural History Society* 38, 151 : August 1935.

The host plants of *Chrysomphalus aurantii* in India are listed, of these a number are of economic importance, although *C. aurantii* is a serious pest in many parts of the World, it is not of any great economic importance in this country.

C. aurantii and the lac insect, *Laccifer lacca*, were both observed parasitic on young grape fruit (*Citrus decumana* var. *grandis*) grafts in the Ranchi District, Chota Nagpur,—this is an example of lac as a pest. Of the sprays used to control *C. aurantii*, a kerosene-soap emulsion whose preparation is described was the most effective, a resin spray proved satisfactory. Lime-sulphur is not recommended as it scorched foliage severely.

A short description of the female scale is given, and certain differences from the normal in the pygidium of the female insect are described, believed to be local variations. Of these that of greatest interest is that the external paraphyse of the second lobe is greatly reduced and may be absent, and that a distinct club-shaped thickening of the cuticle of the dorsal surface arises from the base of the third incisura, whose structure is exactly that of a paraphyse, but whose disposition is abnormal. The pygidium is figured.

The *Aphelinid Aphytis chrysomphali* Mercet was taken resting on a twig infected with *C. aurantii*, this species was also bred from *Aspidiotus orientalis*. This is thought to be first record for India. The *Encyrtid Comperiella bifasciata* How. is a common parasite of *A. orientalis* and is recorded from India for the first time, and is the second species of the genus known to occur in this country, the other being *C. indica* Ramkrishna Ayyar, other *Chalcidoidea* bred from *A. orientalis* were *Physcus* sp. (near *flaviventris* How.) rarely ; *Tetrastichus purpureus* Cam. as a primary and probably also as a secondary parasite, and *Marietta javensis* How. as a hyperparasite of primary *Chalcidoid* parasites of the *Coccid*. (*Author's abstract*)

MYCOLOGICAL NOTES

A DISEASE OF *CYPERUS TEGETIFORMIS* ROXB. CAUSED BY *KAWAKAMIA CYPERI* (MIYABE AND IDETA) MIYABE

BY

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(Received for publication on 30th March 1936)

(With Plate LV)

Cyperus tegetiformis Roxb. is found in low and wet places throughout Bengal, Assam and Bihar, flowering during the rains. It also occurs in China and Japan, and has been cultivated in the Southern States of America. In Bihar it is collected as a fodder, and for some years past a diseased condition of its twigs and leaves has been noticed during the rainy season.

In the early stages the affected culms are bright yellow and with the progress of the disease they become reddish brown. Finally the cells in the infected tissues collapse and the culms wilt. Transverse sections of the affected parts show extensive unseptate mycelium ramifying in the tissues both intra—and intercellularly. Oospores are also occasionally observed. Incubation of affected plants in a damp chamber leads to rapid growth of the cobwebby mycelium, and to development of conidia.

The conidia have a prominent beak, and a peculiar cell which persists as an appendage or pedicel. The beak very easily breaks off leaving the spore with an abrupt flattened apex, and it is rather difficult to get a conidium with the beak attached. The conidia without the beak are 30-70μ in length and 20-35μ in width, the mean being $49\mu \times 21\mu$. These measurements are based on the examination of 200 conidia; the beak was found attached in only fifty-six conidia, and the range of the length was 3-15μ and the mean $5 \cdot 3\mu$.

The fungus is that described by Miyabe from Japan as *Kawakamia Cyperi* [Miyabe, 1904].

It has also been reported as a serious disease by Patterson [1910] in Texas and South Carolina, where *Cyperus* was introduced for the manufacture of matting.

Miyabe gives the maximum size of conidia as $68 \times 32\mu$ whereas Patterson obtained conidia up to $130 \times 30\mu$. The Pusa strain agrees more closely in size of

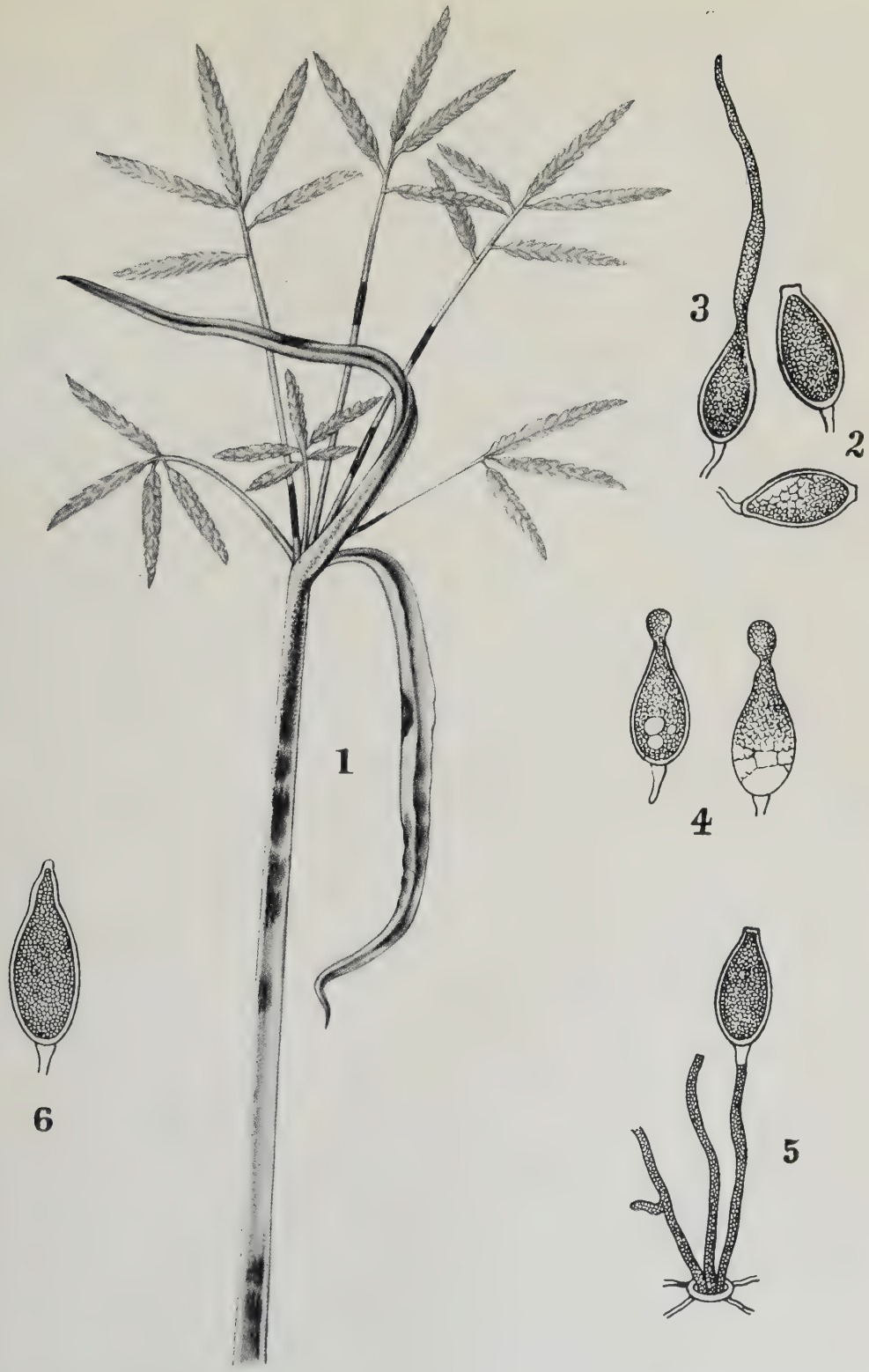

Fig. 1. *Kawakamia* on *Cyperus*
Fig. 2—6. Details of conidia (enlarged).

conidia with the measurements from Japan. Comparison with a herbarium specimen from Texas, however, shows that in general symptoms produced it agrees with the Texas strain.

Fitzpatrick [1930] considers that there is not sufficient reason for separating *Kawakamia* from the genus *Phytophthora*. Wilson [1914], on the other hand, considers it most closely allied to *Basidiophora*. The principal arguments for differentiating *Kawakamia* as a separate genus are the presence of the characteristic, large pedicel and the inability of the fungus to grow on culture media.

This is the first record of *Kawakamia Cyperi* from India.

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A RHIZOCTONIA ON SWEET POTATOES IN BOMBAY

BY

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(Received for publication on 29th April 1936)

(With Plate LVI)

In February 1936 the writer observed in a road-side shop at Kodibag, near Karwar in the Bombay Presidency, several sweet potatoes, *Ipomoea batatas* Poir., that were apparently suffering from disease. The fleshy roots were very much reduced in size and shrivelled and on the surface of their skin, which was red, there were two, three or sometimes four large sclerotia-like bodies such as are seen on the surface of Irish potato tubers suffering from Black Scurf. An examination later of the sweet potatoes offered for sale in the Karwar market indicated that the disease was rather common and that these fetched a very poor price. They came from the neighbouring villages but more information could not be gathered.

With a view to seeing whether the disease was common in the other parts of the Presidency, a visit was paid to the Null bazaar and Crawford markets in the city of Bombay. The sweet potatoes offered for sale were of the white-skinned variety but none of them showed the suspected sclerotia. Whether the roots had been graded and only the better ones sent to the city could not however be ascertained.

The sclerotia when sectioned showed rectangular cells whose walls were thickly incrusted with a black substance. They were rinsed in alcohol, flamed and placed on agar slopes. Hyphae became perceptible in the culture tubes on the third day and profuse mycelial growth on the sixth. After the tenth day, sclerotial formation had started.

The mycelium is light brown, silky, and grows on the walls of the tube. The young hyphae branch freely and fusing hyphae are frequently noticeable. The branches are, as a rule, acute-angled, constricted as they leave the parent hypha and the septum is a little beyond the place of origin. The cells of the hypha are subhyaline, 5 to 10μ in thickness and about 50 to 176μ in length. Sclerotia form slowly in culture, and they are irregular in shape and size. They are addressed, irregularly round, and several of them may coalesce to form a crusty, brittle stromatic mass. They have to be soaked in lacto-phenol for several days before they can be sectioned.

Rhizoctonia solani on sweet potatoes
a—Sclerotia.

The above characters indicated that the fungus is a strain of *Rhizoctonia solani* Kuhn (*Corticium vagum* B. and C.). When compared with cultures of *R. solani* that were in the laboratory, one of which had come from Dr. H. H. Whetzel and another from Mr. S. F. Ashby, no doubt was left regarding the identification.

A *Rhizoctonia*-rot of sweet potato plants has been known for some years in the U. S. A. and Harter and Weimer [1929] mention that sclerotia have been found on the surface of the fleshy roots. How far the sweet potato plants are also affected here by this fungus, it was not possible for the writer to ascertain as there was no crop in the villages round Karwar at the time.

Rhizoctonia solani has not so far been reported on sweet potatoes in India as neither Butler and Bisby [1931] nor Uppal, Patel and Kamat [1935] mention it on this host. In addition to making the sweet potatoes unsaleable, it is possible that the fungus may cause damage to the crop as well.

Diseased specimens of the fleshy roots have been deposited in the mycological herbarium of this Institute. Plate LVI shows three of them with sclerotia on the surface, the position of the sclerotia being marked "*a*".

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REVIEW

A comparative study of certain morphological characters of sugarcane × sorgo hybrids. B. A. Bourne. (*Journal of Agricultural Research*, Vol. **50**, No. 6 : 15th March 1936).

The article gives in some detail the morphological and other characters exhibited by certain intergeneric hybrids between sugarcane and sorghum raised in Florida during 1931-32 and 1932-33.

The studies were made on a population of 128 hybrid plants with the sugarcane variety P. O. J. 2725 as the mother parent ; the male parents employed included (1) Taxas Seeded Ribbon, (2) Early (Kansas) Orange Sorgo, and (3) Sugar Drip Sorgo. The studies reported were chiefly on hybrids with Texas Seeded Ribbon Sorgo.

A rather interesting observation in this article is that " sugarcane stigmas in India have an osmotic pressure very much nearer the optimum for sorgo pollen germination than in either Hawaii or ..... Florida ". This, according to the author, explains the greater ease with which the intergeneric cross between sugarcane and sorghum has been carried out at Coimbatore in India. To ensure success under Florida conditions the sugarcane arrows were covered with a bag from late evening till about 10 A.M. on the succeeding day to prevent reduction of osmotic pressure in the sugarcane stigmatic fluid from rain or dew.

The hybrid plants were found to be intermediate between the two parents in leaf-width and stem-diameter, while in height their average was significantly less than that of either parent. The bud groove on the internode, so characteristic of sorghum, was evident in quite a large number (41 out of 47) of the hybrids. In the inflorescence the sorghum blood was indicated by the frequent presence of the awned IV glume. The hybrid inflorescences exhibited characters, not present in either of the parents such as multiple ovaries and multiple stigmas. The hybrid spikelets were often found to be larger in diameter than those of sugarcane—an obvious influence of sorghum blood. The study includes relative widths of epidermal cells, the distribution of the characteristic short cell groups, the relative number of stomata on the stem epidermis and the presence and distribution of cork and silica cells. The results recorded are of considerable botanical interest. No indication is given of the juice qualities of the hybrids.

The first report of intergeneric hybridization between sugarcane and sorghum was from Coimbatore in 1930. Since then such hybridization has been successfully

effected in Hawaii, Mauritius, Florida and Java. As mentioned by the author, the morphological characters exhibited by the hybrids in Florida are very similar to those described from Coimbatore. The mother parent employed at Coimbatore during the first years was also P. O. J. 2725, but the father parent was *Sorghum Durra* Stapf. The reciprocal cross with sorghum as mother parent was not successful in Florida as also at Coimbatore. (T. S. V.)

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F. J. F. SHAW, C.I.E., D.Sc., A.R.C.S., F.L.S., I.A.S.

OBITUARY

We regret to announce the death of a member of our Editorial Committee, Dr. F. J. F. Shaw, C.I.E., D.Sc., A.R.C.S., F.L.S., I.A.S., Officiating Agricultural Expert, Imperial Council of Agricultural Research, who was the permanent Director of the Imperial Institute of Agricultural Research. The Indian Agricultural Service has lost one of its most distinguished and valued members. Dr. Shaw died at Pusa on the morning of July the 29th. He had gone there on tour to advise regarding some final details of the transfer of the Institute to its new building at Delhi.

Dr. Shaw entered the Indian Agricultural Service in 1910 and was first posted to the Imperial Institute of Agricultural Research as Supernumerary Mycologist. Except for the short time spent at Coimbatore as Mycologist to the Madras Department of Agriculture, he spent the whole of his service at the Pusa Research Institute where he became the Second Mycologist and eventually succeeded Sir Albert Howard as Imperial Economic Botanist when the latter became Director of the Institute of Plant Industry, Indore. Between 1929 and 1934 he was Joint Director for a considerable period and in the latter year he succeeded Dr. McRae as permanent Director of the Institute.

An all-round Botanist with a broad scientific outlook, Dr. Shaw has a very considerable volume of successful research to his credit and his name will always be particularly associated with the progress made in the improvement of Indian tobacco and various oil-seeds. As a post-graduate teacher he achieved reputation throughout India and many officers occupying responsible positions in Provincial Agricultural Departments owe much to his wise guidance and the training in research technique which he gave them. The planning of the new Institute at New Delhi was almost entirely his work and, though he had the satisfaction of seeing the completion of the buildings and of the arrangements for transfer, it was not given to him to enter into residence at the new Institute with which his name will be for ever associated.